MATHEMATICAL MODELING OF HEPATIC AND ADIPOSE INSULIN RESISTANCE IN GIRLS WITH POLYCYSTIC OVARIAN SYNDROME

by

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ABSTRACT

Polycystic Ovarian Syndrome (PCOS) affects 6-10% of women in the United States and is one of the leading causes of infertility. The metabolic phenotype for PCOS includes insulin resistance (IR), and IR may vary with tissue type. The goal of this project is to determine the contributions of hepatic (liver) tissue and adipose (fat) tissue to increased IR in a cohort of adolescent females. For hepatic IR, we use the Labeled Oral Minimal Model (OMM*), originally developed by Dalla Man and colleagues. This differential equations based mathematical model describes the dynamics of glucose and insulin during an oral glucose tolerance test (OGTT) with stable isotope tracers for glucose and glycerol. Parameters for OMM* are used to quantify hepatic IR. We investigate the identifiability of this system and estimate model parameters in 6 subjects. For adipose IR, we propose a novel approach to modeling glycerol dynamics during an OGTT. We investigate the identifiability of this system and develop a numerical approach for estimating model parameters in the same cohort of 6 subjects. We propose the use of model parameters and metrics involving the numerical solution to quantify adipose IR. This work contributes to an improved understanding of tissue-specific IR, which may lead to targeted therapeutics.

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CHAPTER 1
INTRODUCTION

Polycystic Ovarian Syndrome (PCOS) affects 6-10% of women and is the leading cause of infertility in the United States [17]. The metabolic phenotype of PCOS includes higher insulin resistance, type 2 diabetes, nonalcoholic fatty liver disease, and cardiovascular disease, as well as elevated androgens, or male sex hormones [2,7,13,15]. Currently, there is no known cause or cure for PCOS, so treatment is purely symptom relief [14,18,19]. Therefore, gaining a better understanding of tissue specific insulin resistance could suggest a targeted clinical approach for new therapeutics.

The primary metabolic goal of the body is to provide glucose as the energy source for the brain [26]. While fasting, glucose must be produced from endogenous sources. The primary way the brain gets glucose is from the breakdown of glycogen stores in the liver (or hepatic tissue). As a secondary source, glucose can be created from noncarbohydrate carbon sources via gluconeogenesis. This process begins with the break down of triglycerides in adipose tissue, or fat tissue. This breakdown is called lipolysis. Lipolysis releases free fatty acids (FFA) and glycerol, which are substrates for gluconeogenesis. During fasting, FFA is the main energy source for all tissues except the brain and red blood cells [26].

Postprandial metabolism is very different since exogenous glucose is readily available. In this state, the brain and all other tissues have access to exogenous glucose, so endogenous glucose sources need to be shut down. The liver needs to stop releasing glucose and should synthesize glycogen (energy storage form of glucose). Also, lipolysis needs to be suppressed. Adipose tissue takes up FFA and glycerol to synthesize triglycerides. In addition to limiting endogenous processes, excess glucose needs to be stored. The liver takes up glucose in a concentration dependent manner, and adipose tissue (as well as muscle) takes up glucose in an insulin mediated way.

Insulin is the main hormone responsible for controlling the processes during the postprandial state, as well as the switching between the two states. Insulin is released from the pancreas in response to high glucose levels. During the postprandial state, insulin's primary role is to promote excess glucose clearance from plasma by signaling hepatic tissue to synthesize glycogen and stop glucose production, as well as suppress lipolysis in adipose tissue.

One of the most common diseases related to PCOS is Type 2 diabetes. Diabetes Mellitus is a disease that is characterized by inadequate production or utilization of insulin. Type 1 diabetes (also called juvenile diabetes and insulin-dependent diabetes) is marked by impaired insulin production by the pancreas, so patients with Type 1 diabetes need additional insulin as their bodies do not produce enough to carry out the basic functions required of insulin. Type 2 diabetes patients produce insulin but may require high levels of insulin since their tissue is resistant to insulin. If insulin is not working properly, the liver continues to release glucose, muscles do not uptake glucose efficiently, and lipolysis continues in adipose tissue, releasing FFA and glycerol. **Insulin resistance** (IR) occurs when higher than normal levels of insulin are required to stop glucose release from the liver, and to sufficiently suppress adipose tissue releasing FFA and glycerol. Therefore, a precursor to type 2 diabetes is increased IR. PCOS patients have high levels of IR, which often leads to type 2 diabetes. Other forms of diabetes include gestational diabetes, surgically induced diabetes, and chemically induced diabetes. Gestational diabetes develops during pregnancy caused by increased hormones. This form of diabetes usually subsides after delivery. Surgically induced diabetes occurs when surgery is performed on the pancreas that results in permanent or temporary damage to the process that releases insulin. Chemically induced diabetes is a result of medications that increase blood sugar levels.

Isotope tracers represent a key tool for probing insulin resistance. Isotope tracers are molecules that contain atoms with a different number of neutrons than the tracee. A tracee is the naturally occurring tracer counterpart. By measuring tracers and tracees concurrently, exogenous and endogenous contributions can be differentiated. The main assumption used in tracer studies is that the tracer is indistinguishable within the naturally occurring processes with respect to the properties of the tracee [9]. Isotopes can either be radioactive or stable. Radioactive isotopes have an unstable nucleus that spontaneously decomposes by releasing energy, and are considered massless. Stable isotopes have a stable nucleus and cannot be considered massless. Previously, radioactive isotopes were the primary tracer used since having no mass was easier to deal with, but these tracers cannot be used on children, pregnant women, or replicated in the same subjects [5]. Therefore, stable tracers are now used more often.

The main goal of the project is to achieve a better understanding of tissue specific insulin resistance in girls with PCOS. The study is designed to measure glycerol and glucose during an oral glucose tolerance test (OGTT) by using three stable tracers: glycerol tracer [$^2H_5$]glycerol intravenously (IV), and glucose tracers [6,6-$^2H_2$]glucose (IV) and [1-^{13}C]glucose (oral). The glycerol tracer is used to quantify adipose insulin resistance, whereas the glucose tracers are used to quantify hepatic insulin resistance.

The glucose tracers will give information on hepatic IR. The IV glucose tracer will be infused before the oral load is given in order to measure the endogenous glucose production. The oral tracer will be used to show the exogenous glucose. Together, these values will show if endogenous glucose production is suppressed when exogenous glucose is present. The experimental design and concepts are described by Dalla Mann et. al. [11]. If endogenous glucose production is not suppressed, then this would imply hepatic IR.

Glycerol tracer should give information on adipose IR since effective insulin action on adipose tissue should suppress glycerol production. In other words, adipose IR would present itself as higher than normal insulin levels with expected glycerol concentrations throughout the experiment. Historically, glycerol has mainly been used as a way to measure whole body lipolysis [5, 21]; however, glycerol rate of appearance (Ra), may not be an ideal measure of whole body lipolysis since glycerol and FFA might have a more complicated relationship than the commonly assumed 3:1 ratio during lipolysis release [1]. Therefore, glycerol Ra might not tell all the information needed about FFA to create a measure of whole body lipolysis. However, Jensen's research [1] was focused on whole body lipolysis, whereas this project is focused on tissue specific insulin resistance. In the case of adipose insulin resistance in PCOS, a qualitative result of markedly higher insulin levels with normal glucose levels during the OGTT would imply adipose IR in PCOS. On the other hand, no change between the obese PCOS subjects and the obese control would imply either inconclusive results on the status of adipose IR or adipose IR is not present in PCOS.

CHAPTER 2
BACKGROUND

This chapter will discuss the experimental design and Steele's non-steady-state rate of appearance.

2.1 EXPERIMENTAL DESIGN

We used a stable isotope tracer protocol to measure glucose and glycerol during an oral glucose tolerance test (OGTT). A detailed explanation of this protocol is as follows.

Participants:

Six participants were recruited from pediatric clinics at the Children's Hospital Colorado for a prospective, cross-sectional study. Participants included obese youth (BMI $\geq$ 95th percentile for age) without type 2 diabetes. All participants were sedentary (defined as less than 3 hours per week of exercise, verified by standardized 3-day activity recall, and 7-day accelerometer recording, Actigraph, Pensacola, FL), had achieved Tanner Stage 5 in puberty[1] (as assessed by physical exam by a pediatric endocrinologist, Kristen J. Nadeau or Melanie Cree-Green). Anemia[2] was ruled out in all subjects prior to study. Exclusionary medications included antihypertensives, lipid lowering agents, oral steroids, atypical antipsychotics and metformin. This study was approved by the University of Colorado Anschutz Medical Campus Institutional Review Board and the Children's Hospital of Colorado Scientific Advisory Review Committee. Parental informed consent and participant assent was obtained from all participants less than 18 years old and participant consent from those aged 18 years and above.

Overall study design:

Volunteers underwent a screening visit to ensure eligibility and then a study visit with an overnight stay. Three days prior to the overnight admission, participants consumed 3 days of an isocaloric diet (55% carbohydrate, 15% protein, 30% fat) and refrained from physical activity.

Tracer infusion protocol:

Baseline blood samples (background enrichment of glucose and concentrations of glucose, insulin)

[1] The Tanner Scale categorizes physical development of children, adolescents, and adults primarily based on external sex characteristics. Tanner Stage 5 is the final stage on the scale.

[2] Anemia is a condition marked by a deficiency of red blood cells or of hemoglobin in the blood.

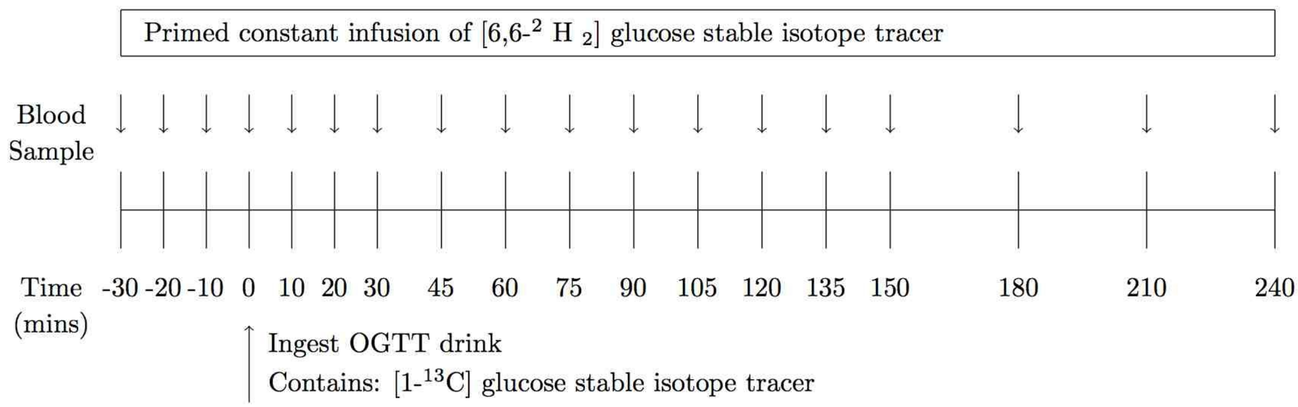

Figure 2.1: Protocol Timeline

were obtained at approximately 6 AM and then a constant infusion of glucose stable isotope was started. A bolus of 4.5 mg/kg [6,6-$^2H_2$]glucose (Isotec, Miamisville, IA), followed by a continuous infusion at 0.03 mg/kg/min [6,6-$^2H_2$]glucose was started and continued throughout the study. During the last 30 minutes of the fasted period, 4 samples, each 10 minutes apart were drawn for glucose and insulin concentrations, as well as for glucose tracer enrichments [16] to assure baseline values are attained and constant. The drink (Glucola) consisted of a standard 75 grams of glucose with an additional 40 mg/kg of $[1-^{13}C]$glucose, and it was consumed in less than 90 seconds. Once the drink was consumed, samples were collected initially every 10 min for the first 30 minutes, then every 15 min until 2 hours post drink, then every 30 min for the remainder of the study; *a priori* knowledge of how glucose appears in the blood system (Ra) following ingestion suggests that glucose will change a lot initially then taper off. Therefore, to optimize the experimental characterization of glucose Ra, we take finer measurements initially, and coarser measurements towards the end.

Sample analysis:

Analysis of [6,6-$^2H_2$] glucose was done using a modification of the negative ion chemical ionization gas chromatography mass spectrometry as described previously [3, 4, 16]. In addition to analysis of the 242/244 fragments produced with electron ionization, the 331, 332, and 333 fragments produced via chemical ionization were also measured [26]. Whole blood glucose concentrations were determined via modified glucose oxidase-based method on a StatStrip®hospital glucose monitoring system (Nova Biomedical, Waltham, MA), which has been demonstrated previously to be equivalent with a YSI 2300 STAT Plus^{TM} (YSI, Inc, Yellow Springs, OH) glucose analyzer [20]. Serum insulin concentration was determined with radioimmunoassay (Millipore, Billerica, MA).

Tracer calculations:
All isotopic enrichments were corrected for background enrichments. The glucose Ra was calculated using Steele equations [24, 25], modified for stable isotopes (§2.2). To account for the possibility of the $[1 -^{13} C]$glucose, label being recycled into the 1,2,5 or 6 position of the glucose, the contribution of $[1 -^{13} C]$glucose, recycling was calculated for each subject with the following formula

$$CI - (EI/3),$$

where CI represents the 333 fragment concentration, and EI is the 244 fragment concentration [26].

2.2 STEELE'S RA

Steele's non-steady-state calculation of rate of appearance (Ra) is the most commonly used model-independent estimate for substrate Ra. Rate of appearance is the entry of tracer into the pool, which can occur at a single site of entry or an aggregate of multiple locations. For example, during the steady-state (fasting), the only point of origin of glucose Ra is the liver; by contrast, adipose tissue releases glycerol from multiple locations (fat tissue all over the body) so glycerol Ra is the sum of all contributions from all of these points [26].

To use Steele's Ra, we assume a single pool structure defined to be "a relatively homogeneous physical entity within which mixing is rapid" [26]. This assumption is valid for our models because blood behaves as a homogeneous rapidly mixing substance. We also assume that no tracer reenters the system (e.g., we assume glucose tracer does not reenter the pool from the liver).

Now we will derive Steele's non-steady-state Ra formula. Define $E(t)$ as the enrichment of the plasma sample at time t. Let $q(t)$ be the amount of tracer at time t and $Q(t)$ be the amount of tracee at time t. Then $E(t) = \frac{q(t)}{Q(t)}$, by definition. Therefore,

$$q(t) = E(t)Q(t) \tag{2.1}$$

and

$$\frac{dq(t)}{dt} = E(t)\frac{dQ(t)}{dt} + Q(t)\frac{dE(t)}{dt}. \tag{2.2}$$

The change in tracee over time is the rate at which tracee appears into the pool minus the rate at which it leaves the pool, or

$$\frac{dQ(t)}{dt} = Ra - Rd. \tag{2.3}$$

Lastly, the change in tracer over time is the rate at which it is infused (F) minus the rate at which it leaves the pool ($Rd \cdot E$), given by

$$\frac{dq(t)}{dt} = F - Rd \cdot E. \tag{2.4}$$

Therefore, substituting (2.3) and (2.4) into (2.2), we have

$$F - Rd \cdot E(t) = E(t)(Ra - Rd) + Q(t)\frac{dE(t)}{dt}. \tag{2.5}$$

Solving for Ra, we obtain

$$Ra(t) = \frac{F - Q(t)\frac{dE(t)}{dt}}{E(t)}. \tag{2.6}$$

However, data suggests that glucose does not behave like an ideal pool in the non-steady-state. To account for this, Steele included the idea that a fraction of the total pool mix rapidly [26]. This means we define $Q(t) = pV \cdot C(t)$ where p is the pool fraction, V is the volume, and $C(t)$ is the concentration at time t. This gives us Steele's Ra:

$$Ra(t) = \frac{F - pV \cdot C(t)\frac{dE(t)}{dt}}{E(t)}. \tag{2.7}$$

In practice, at each time point i, we average the concentration over two time points, estimate the enrichment derivative using two consecutive time points, and average the enrichment from two time points:

$$Ra(i) = \frac{F - pV[(C(i) + C(i+1))/2][(E(i+1) - E(i))/(t(i+1) - t(i)]}{(E(i) + E(i+1))/2}. \tag{2.8}$$

It is important to note that Steele's inclusion of the proportion of the volume that mixes rapidly, pV, is a "correction factor" which corrects for the fact that most metabolic processes are too complex to be modeled by a single pool model. This factor relaxes the single pool assumptions and allows for more complicated systems to use Steele's Ra. We take glucose pV to be 180 ml/kg [26].

Additionally, we can estimate the proportion of Ra coming from the meal (Ra_{meal}) by multiplying Steele's Ra by the glucola enrichment. Recall that glucola is the tracer from the drink ([1-^{13}C]glucose; see §2.1). We measure the total glucola enrichment at the beginning of the experiment (E_D), and we measure the plasma enrichment of [1-^{13}C]glucose (E_P). Also, we measure the total glucose at each time point. Then, the glucola contribution is a proportion of the total measured glucose, given by

$$G_{\text{meal}} = \text{glucola contribution} = \frac{E_P}{E_D} \cdot \text{ total measured glucose.} \tag{2.9}$$

Ra glucose from the glucose drink (Ra_{meal}) and endogenous glucose production (Ra_{ENDO}) were calculated using the following equations:

$$\begin{aligned} Ra_{meal} &= Ra\left(\frac{E_P}{E_D}\right) \\ Ra_{ENDO} &= Ra - Ra_{EXO} \end{aligned}$$

where E_D and E_P are the enrichments of the [1-^{13}C]glucose in the drink and plasma, respectively.

Glycerol pV has been estimated to be 0.027 L/kg [26].

CHAPTER 3
GLUCOSE (HEPATIC IR)

The basic model for glucose and insulin dynamics that is most commonly used is the Minimal Model (MM) originally introduced by Cobelli et al. [10]. MM is a one compartment model that describes subject-specific glucose and insulin dynamics. This model was extended to describe glucose and insulin dynamics during an OGTT, called the Oral Minimal Model (OMM*), by Dalla Man et al. [11]. This chapter will start by deriving OMM* by first explaining MM, then investigate identifiably of the model parameters, and end with comparing model parameters, as well as other metrics, between the Obese Control group and the Obese PCOS group in order to determine if hepatic IR occurs in girls with PCOS.

3.1 MINIMAL MODEL DERIVATION

In order to derive MM, we will start by considering the fasting state (see Figure 3.1). As discussed in the introduction, glucose is the main fuel supply for the brain. During the fasting state, glucose is released from the liver via the breakdown of glycogen stores (Ra in the picture), and it is also taken up by the liver (R_{dL}). This cycle of glucose release and uptake by the liver is called net hepatic glucose production (NHGP). Glucose is also utilized by the periphery (R_{dP}). Note that the subscripts L and P denote liver and periphery, respectively.

Insulin is the primary mechanism for controlling glucose dynamics. The equation describing insulin action on glucose is given by

$$\frac{d\hat{I}(t)}{dt} = k_2[I(t) - I_b] - k_3\hat{I}(t), \qquad \hat{I}(0) = 0$$

where $\hat{I}$ is the remote insulin acting on glucose (μU/ml), I is the plasma insulin concentration (μU/ml), I_b is the basal value (μU/ml), and k_2, k_3 are constant nonnegative parameters (min^{-1}).

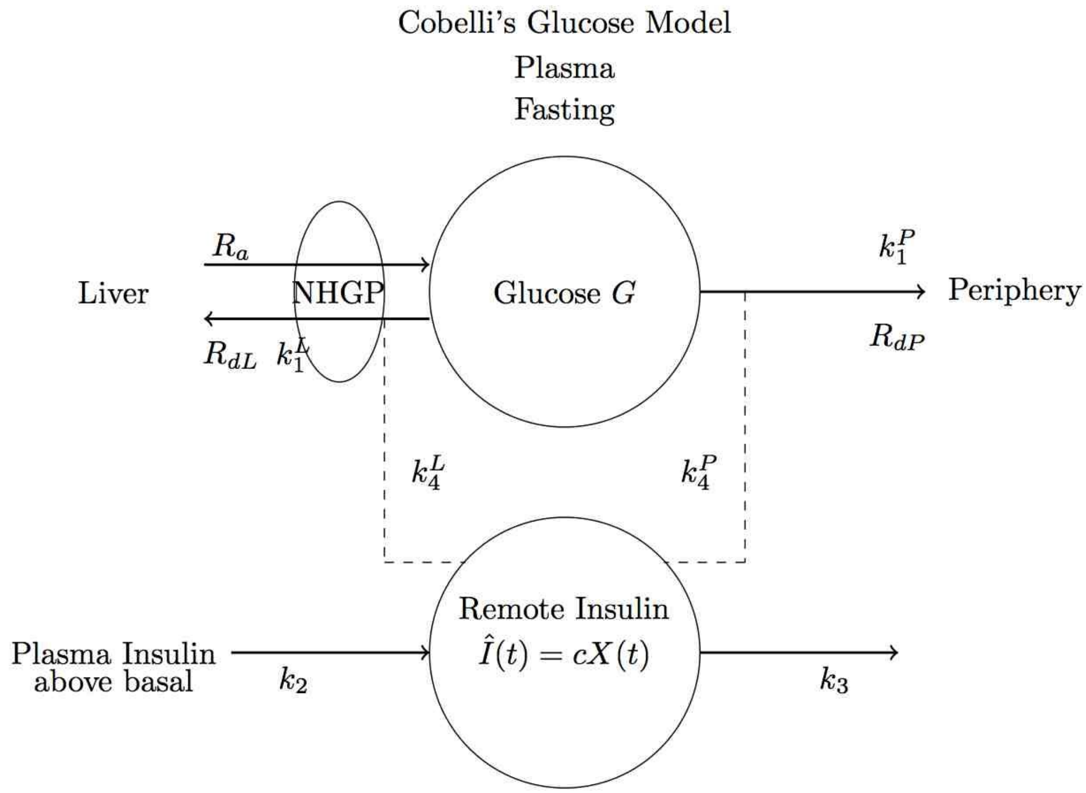

Figure 3.1: The Minimal Model - Unlabeled Glucose
Rate parameters k. Dashed lines represent control actions; solid lines represent material fluxes.

To derive the equation for total glucose, we start with the mass balance principle:

$$\frac{dG(t)}{dt} = \frac{Ra(t) - Rd(t)}{V}, \quad G(0) = G_0$$

where G is the plasma glucose concentration (mg/dl/min), V is the distribution volume (dl/kg), Ra is the rate of appearance of glucose (mg/kg/min) and Rd is the rate of disappearance of glucose (mg/kg/min). Note that $Rd = R_{dL} + R_{dP}$. Therefore, we have

$$\frac{dG(t)}{dt} = \frac{Ra(t) - R_{dL}(t) - R_{dP}(t)}{V} = \frac{NHGP(t) - R_{dP}(t)}{V}, \tag{3.1}$$

since $Ra = R_{aL}$ in the fasting state. Cobelli et al. [10] introduced the following functional forms for NHGP and R_{dP}:

$$NHGP(t) = B_0 - k_4^L \hat{I}(t) G(t) - k_1^L G(t) \tag{3.2}$$

and

$$R_{dP}(t) = k_1^P G(t) + k_4^P G(t)\hat{I}(t) \tag{3.3}$$

where B_0 is extrapolated NHGP at zero glucose (mg/kg/min), and $k_1^L, k_1^P, k_4^L, k_4^P$ are constant nonnegative parameters with units V/min for k_1^L, k_1^P and (ml/μ U) V/min for k_4^L, k_4^P. Substituting equations (3.2) and (3.3) into equation (3.1), we have

$$\frac{dG(t)}{dt} = \frac{-(k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t) + B_0}{V}. \tag{3.4}$$

Finally, the original minimal model for unlabeled glucose fasting dynamics is given by

$$\left.\begin{aligned} \frac{dG(t)}{dt} &= \frac{-(k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t) + B_0}{V}, & G(0) = G_0 \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), & \hat{I}(0) = 0. \end{aligned}\right\} \tag{3.5}$$

3.1.1 PARAMETER REDUCTION FOR MM

The minimal model of unlabeled glucose fasting dynamics is system unidentifiable. This is clear from the terms $(k_1^L + k_1^P)$ and $(k_4^L + k_4^P)$. For more information, see §3.3. In order to reduce the number of parameters in the model, we define the following parameter combinations based on their biological role:

$$\left.\begin{aligned} X(t) &= \frac{(k_4^L + k_4^P)}{V}\hat{I}(t) \\ p_1 &= k_1^L + k_1^P \\ p_2 &= k_3 \\ p_3 &= \frac{k_2(k_4^L + k_4^P)}{V} \\ p_4 &= B_0. \end{aligned}\right\} \tag{3.6}$$

These parameter combinations maintain biological implications. For example, p_1 contains the glucose clearance parameters, and p_3 is the parameter describing remote insulin effect on glucose clearance. Note that in the basal steady state,

$$\frac{dG(t)}{dt} = 0, \qquad G(t) = G_b$$
$$\frac{dX(t)}{dt} = 0, \qquad X(t) = 0_b,$$

which gives us $p_4 = p_1 G_b$ as $\dot{G}(t) = 0$ implies $-[p_1 + 0_b]G_b + p_4 = 0$. Further, define $S_G = \frac{p_1}{V}$, which is the fractional glucose effectiveness, a measure of the ability of glucose to promote glucose disposal and inhibit NHGP. Substituting into the glucose equation in (3.23), we obtain

$$\begin{aligned}\frac{dG(t)}{dt} &= -\frac{p_1 G(t)}{V} - X(t)G(t) + \frac{p_1 G_b}{V} \\ &= -[S_G + X(t)]G(t) + S_G G_b.\end{aligned}$$

For the insulin equation, we have

$$X(t) = \frac{(k_4^L + k_4^P)}{V}\hat{I}(t),$$

which implies

$$\begin{aligned}\frac{dX(t)}{dt} &= \frac{(k_4^L + k_4^P)}{V}\frac{d\hat{I}(t)}{dt} \\ &= \frac{(k_4^L + k_4^P)}{V}\left[k_2[I(t) - I_b] - k_3\hat{I}(t)\right] \\ &= \frac{1}{V}k_2(k_4^L + k_4^P)[I(t) - I_b] - k_3\frac{1}{V}(k_4^L + k_4^P)\hat{I}(t) \\ &= p_3[I(t) - I_b] - k_3 X(t) \\ &= p_3[I(t) - I_b] - p_2 X(t),\end{aligned}$$

with initial condition

$$X(0) = \frac{(k_4^L + k_4^P)}{V}\hat{I}(0) = \frac{(k_4^L + k_4^P)}{V}(0) = 0.$$

Thus, the final form of the minimal model for unlabeled glucose is given by

$$\left.\begin{aligned}\frac{dG(t)}{dt} &= -[S_G + X(t)]G(t) + S_G G_b, \qquad & G(0) = G_0 \\ \frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), \qquad & X(0) = 0.\end{aligned}\right\} \tag{3.7}$$

3.1.2 MM FOR LABELED GLUCOSE

Next, we will consider the dynamics for labelled glucose introduced directly to the system by injection. In contrast to the assumption for unlabeled glucose, we assume that labelled glucose tracer is not recycled back into the system. Let labelled glucose be denoted by G^*. Then,

$$\frac{dG^*(t)}{dt} = \frac{-R_d^*(t)}{V} = \frac{-[R_{dL}^*(t) + R_{dP}^*(t)]}{V},$$

where the asterisk denotes tracer.

The indistinguishability principle implies the relationship

$$\frac{R_{dP}^*}{G^*} = \frac{R_{dP}}{G},$$

which gives

$$R_{dP}^*(t) = \frac{G^*(t)}{G(t)}\left[k_1^P G(t) + k_4^P G(t)\hat{I}(t)\right] = k_1^P G^*(t) + k_4^P \hat{I}(t)G^*(t).$$

Hence,

$$\frac{dG^*(t)}{dt} = \frac{-R_{dL}^*(t) - [k_1^P + k_4^P \hat{I}(t)]G^*(t)}{V}.$$

Following [10], we assume $R_d^*(t)$ has the same functional dependence on $G^*(t)$ and $\hat{I}(t)$ as $R_{dP}^*(t)$. Then, the minimal model for labeled glucose tracer dynamics is

$$\left.\begin{aligned} \frac{dG^*(t)}{dt} &= \frac{-[(k_1^L + k_1^P) + (k_4^L + k_4^P)\hat{I}(t)]G^*(t)}{V}, \quad G^*(0) = G_0^* \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), \qquad \hat{I}(0) = 0. \end{aligned}\right\} \tag{3.8}$$

Again, this model is system unidentifiable (§3.3.1). Using the parameter combinations in equation (3.6), we have the final form of the minimal model for glucose tracer dynamics:

$$\left.\begin{aligned} \frac{dG^*(t)}{dt} &= -[S_G^* + X^*(t)]G^*(t), \qquad G^*(0) = G_0^* \\ \frac{dX^*(t)}{dt} &= p_3^*[I(t) - I_b] - p_2^* X^*(t), \quad X^*(0) = 0. \end{aligned}\right\} \tag{3.9}$$

The equations in (3.7) and (3.9) are the equations for the complete minimal model. This concludes the minimal model derivation.

3.2 ORAL MINIMAL MODEL DERIVATION

The derivation for OMM* is similar to the previous derivations, but we must account for glucose entering the system from a meal (see Figure 3.2). During the postprandial state, insulin shuts off hepatic tissue release of glucose and promotes glucose uptake by the liver and periphery. When hepatic glucose release is suppressed, the only glucose entering the system is from the meal (Ra_{meal}). Thus, in the postprandial state, NHGP is limited to uptake and we may assume that no glucose tracer is recycled back into the plasma by the liver.

First, we will consider total glucose. Following a similar method to MM, we have

$$\begin{aligned}\frac{dG(t)}{dt} &= \frac{Ra(t) - Rd(t)}{V} \\ &= \frac{Ra_{\text{meal}}(t) + R_{aL}(t) - R_{dL}(t) - R_{dP}(t)}{V} \\ &= \frac{Ra_{\text{meal}}(t) + NHGP(t) - R_{dP}(t)}{V}.\end{aligned}$$

Using the same functional description for $NHGP$ and R_{dP} as outlined in equations (3.2) and (3.3), we have

$$\frac{dG(t)}{dt} = \frac{Ra_{\text{meal}}(t) + B_0 - (k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t)}{V}.$$

Using the parameters in equation (3.6), we get

$$\begin{aligned}\frac{dG(t)}{dt} &= \frac{Ra_{\text{meal}}(t)}{V} + \frac{p_1 G_b}{V} - \frac{p_1 G(t)}{V} - X(t)G(t) \\ &= \frac{Ra_{\text{meal}}(t)}{V} + S_G G_b - S_G G(t) - X(t)G(t) \\ &= \frac{Ra_{\text{meal}}(t)}{V} + S_G G_b - [S_G + X(t)]G(t).\end{aligned}$$

The insulin equation is the same as in (3.7). Therefore,

$$\left.\begin{aligned}\frac{dG(t)}{dt} &= -[S_G + X(t)]G(t) + S_G G_b + \frac{Ra_{\text{meal}}(t)}{V}, \quad & G(0) = G_b \\ \frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), & X(0) = 0\end{aligned}\right\} \tag{3.10}$$

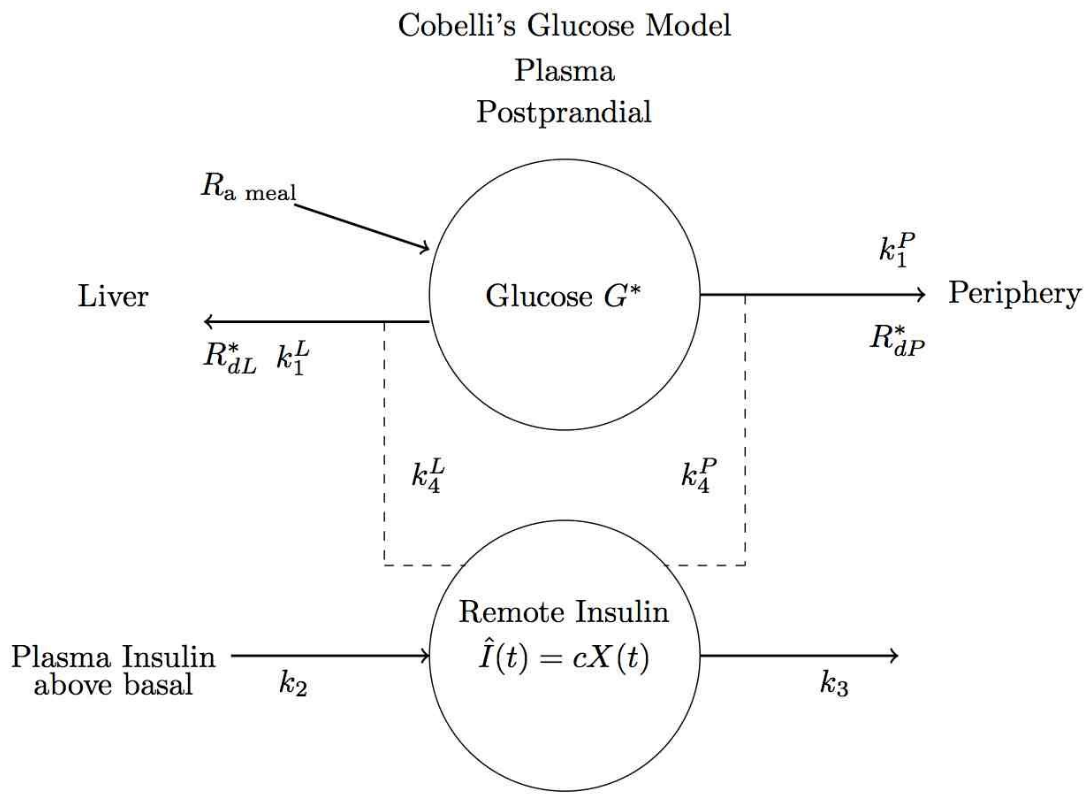

Figure 3.2: The Oral Minimal Model - Labeled Glucose

is the oral minimal model for unlabeled glucose. For this system, X represents the insulin action on glucose disposal and production, and S_G is the fractional glucose effectiveness, a measure of the ability of glucose to promote glucose disposal and inhibit NHGP [11].

In order to isolate insulin action on glucose disposal from glucose production, we will consider labeled glucose introduced to the system from a meal, denoted G_{meal}. Asterisks denote quantities associated with labeled glucose. Using a similar approach, we have

$$\frac{dG_{\text{meal}}(t)}{dt} = \frac{Ra_{\text{meal}}(t) - Rd_{\text{meal}}(t)}{V^*}, \quad G_{\text{meal}}(0) = 0,$$

where Rd_{meal} is the rate of disappearance of oral glucose. Following [11], we assume the same functional dependence for Rd_{meal} as Rd, which implies

$$Rd_{\text{meal}}(t) = (k_1^P + k_1^L)G_{\text{meal}}(t) + (k_4^P + k_4^L)\hat{I}(t)G_{\text{meal}}(t).$$

This gives

$$\frac{dG_{\text{meal}}(t)}{dt} = \frac{Ra_{\text{meal}}(t) - (k_1^P + k_1^L)G_{\text{meal}}(t) - (k_4^P + k_4^L)\hat{I}(t)G_{\text{meal}}(t)}{V^*}. \tag{3.11}$$

Using the parameters in equation (3.6), we have the labeled oral minimal model

$$\left.\begin{aligned} \frac{dG_{\text{meal}}(t)}{dt} &= -[S_G^* + X^*(t)]G_{\text{meal}}(t) + \frac{Ra_{\text{meal}}(t)}{V^*}, \quad & G_{\text{meal}}(0) = 0 \\ \frac{dX^*(t)}{dt} &= p_3^*[I(t) - I_b] - p_2^* X^*(t), & X^*(0) = 0. \end{aligned}\right\} \tag{3.12}$$

For this model, X^* is insulin action on glucose disposal, and S_G^* is the fractional glucose effectiveness measuring glucose ability to promote glucose disposal [11]. Including equations (3.10) and (3.12), we have derived the complete oral minimal model.

The following parameter combinations are used to quantify insulin sensitivity [11]:

$$S_I = \frac{p_3}{p_2}V \tag{3.13}$$

describes total insulin sensitivity and

$$S_I^* = \frac{p_3^*}{p_2^*}V \tag{3.14}$$

describes insulin sensitivity on glucose disposal. This second measure quantifies hepatic insulin resistance since glucose disposal mediated by hepatic tissue.

3.3 IDENTIFIABILITY ANALYSIS FOR THE MINIMAL MODEL

For this section, we will use the Taylor Expansion method in order to determine the identifiability of the model parameters. The Taylor Expansion Method[3] for determining identifiability

[3] Also called the Power Series Expansion method

considers the system

$$\begin{aligned} \dot{x}(t) &= f(x(t), u(t), t, \vec{p}), \qquad x(t) \in \mathbb{R}^n, \quad t \in [0, T] \\ y(t) &= h(x(t), \vec{p}) \end{aligned} \tag{3.15}$$

where $x(t)$ is the state vector, $u(t)$ is the control (input) vector, $y(t)$ is the observation (measured) vector, and $\vec{p}$ is the parameter vector. Assume $f(x(\cdot), u(\cdot), \cdot, \vec{p})$ has infinitely many derivatives with respect to time and the input and state vector components, $u(\cdot)$ has infinitely many time derivatives, and $h(x(\cdot), \vec{p})$ has infinitely many derivatives with respect to the state vector components [22]. Due to this assumption, $x(\cdot), y(\cdot)$ also have infinitely many time derivatives [22]. Define

$$a_k(\cdot) = y^{(k)}(\cdot), \quad k = 0, \ldots, \infty \tag{3.16}$$

since y is a unique function of time, a_k, for $k = 0, \ldots, \infty$, are unique as well [22]. Pohjanpalo [22] explains

> *It is sufficient for the identifiability of the system* (3.15) *that the set of equations*
>
> $$h^{(k)}(x(0), \vec{p}) = a_k(0), \quad k = 0, \ldots, \infty \tag{3.17}$$
>
> *have a unique solution for* $\vec{p}$.

From (3.17), it is possible to determine if a parameter value is uniquely identifiable (unique solution for p_i), identifiable (finite number of solutions for p_i), or unidentifiable (infinitely many solutions for p_i) [8].

3.3.1 LABELED MINIMAL MODEL

Recall the original labeled glucose minimal model (see §3.1 for details):

$$\left.\begin{aligned} \frac{dG^*(t)}{dt} &= \frac{-[(k_1^L + k_1^P) + (k_4^L + k_4^P)\hat{I}(t)]G^*(t)}{V}, \quad G^*(0) = G_0^* \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), \qquad \hat{I}(0) = 0 \\ y(t) &= G^*(t). \end{aligned}\right\} \tag{3.18}$$

We will use the Taylor Expansion method for identifiability as described in §3.3. This means we will assume $G^*(t), \hat{I}(t)$ have infinitely many derivatives with respect to time and state vector components. For this model, the parameter vector is $\vec{p} = [k_1^L, k_1^P, k_2, k_3, k_4^L, k_4^P, V]^T$. Let $y(t)$ denote the observed glucose concentration at t. Since this method is a local analysis, we will consider the identifiability about $t_0 = 0$. First, we have

$$y(t) = G^*(t),$$

which gives

$$y(0) = G^*(0) = G_0^*.$$

Therefore, G_0^* is a known value. Note that this value is known from the basal data. Taking the first derivative, we get

$$\begin{aligned}
\dot{y}(t) &= \frac{dG(t)}{dt} = \frac{-[(k_1^L + k_1^P) + (k_4^L + k_4^P)\hat{I}(t)]G^*(t)}{V} \\
\dot{y}(0) &= \frac{-[(k_1^L + k_1^P) + (k_4^L + k_4^P)\hat{I}(0)]G^*(0)}{V} \\
&= \frac{-(k_1^L + k_1^P)G_0^*}{V}.
\end{aligned}$$

From here, we obtain the relationship

$$-\frac{\dot{y}(0)}{G_0^*} = \frac{k_1^L + k_1^P}{V}.$$

The left hand side is a known value, but there exists an infinite combination for the parameters that satisfy the right hand side. Therefore, this model is <u>system unidentifiable</u>.

We will now consider the final form of the labeled glucose minimal model. Recall from §3.1, the model uses equation (3.18) with the new parameter combinations

$$\left.\begin{aligned} X(t) &= \frac{(k_4^L + k_4^P)}{V}\hat{I}(t) \\ p_1 &= k_1^L + k_1^P \\ p_2 &= k_3 \\ p_3 &= \frac{k_2(k_4^L + k_4^P)}{V} \\ p_4 &= B_0 \\ S_G &= \frac{p_1}{V}. \end{aligned}\right\} \tag{3.19}$$

to obtain

$$\left.\begin{aligned} \frac{dG^*(t)}{dt} &= -[S_G + X(t)]G^*(t), && G^*(0) = G_0^* \\ \frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), && X(0) = 0 \\ y(t) &= G^*(t) \\ z(t) &= I(t). \end{aligned}\right\} \tag{3.20}$$

For (3.20), the parameter vector is $\vec{p} = [S_G, p_2, p_3]^T$, so the number of model parameters has been reduced from 7 to 3. Again, we will let $y(t)$ denote the observed glucose concentration at t. Also, assume insulin concentration is also measured, and let $z(t)$ denote the observed insulin concentration at t. We assume I_b is known. since the protocol allows for basal measurements ($I(-20), I(-10)$ are known).

We will consider the identifiability about $t_0 = 0$. Technically, we will consider t as $t \to t_0^+$. Before we begin the identifiability analysis, recall the General Leibniz Rule:

If f and g are n-times differentiable functions, then the product fg is also differentiable and its n-th derivative is given by

$$(fg)^{(n)} = \sum_{k=0}^{n} \binom{n}{k} f^{(k)} g^{(n-k)} \tag{3.21}$$

where $\binom{n}{k} = \dfrac{n!}{k!(n-k)!}$ is the binomial coefficient.

The Leibniz Rule applies to $y(t)$ since we assume $G^*(t), \hat{I}(t)$ have infinitely many derivatives with respect to time and state components. Thus, we can find an expression for any derivative of

$y(t)$:

$$\begin{aligned}
y(t) &= G^*(t) \\
y'(t) &= \frac{dG^*}{dt} = -S_G G^*(t) - X(t)G^*(t) \\
y''(t) &= -S_G \frac{dG^*(t)}{dt} - X(t)\frac{dG^*(t)}{dt} - \frac{dX(t)}{dt}G^*(t) \\
&\vdots
\end{aligned}$$

$$y^{(n)} = -S_G \frac{d^{(n-1)}G^*(t)}{dt^{(n-1)}} - \sum_{k=0}^{n-1} \binom{n-1}{k} \frac{d^{n-1-k}X(t)}{dt^{n-1-k}} \frac{d^k G^*(t)}{dt^k}, \qquad n \geq 2. \tag{3.22}$$

Note that the inclusion of insulin adds additional information: $z(t) = I(t) \implies z(0) = I(0) = I_0$ is a known value; $z^{(n)}(t) = I^{(n)}(t)$ is known. We will focus on the observed labeled glucose. By definition, we have

$$y(t_0^+) = G^*(t_0^+) = \lim_{t \to 0} G^*(t) = G_0^*,$$

so G_0^* is a known value. Next,

$$\begin{aligned}
y'(t_0^+) &= -S_G G^*(t_0^+) - X(t_0^+)G^*(t_0^+) \\
&= \lim_{t \to 0}[-S_G G^*(t) - X(t)G^*(t)] \\
&= -S_G G^*(0) - X(0)G^*(0) \\
&= -S_G G_0^*.
\end{aligned}$$

Solving for S_G gives the relationship

$$S_G = -\frac{y'(0)}{G_0^*}$$

so S_G is uniquely identifiable. The second derivative gives

$$\begin{aligned}
y''(t_0^+) &= \lim_{t \to 0} \left[-S_G \frac{dG^*(t)}{dt} - X(t)\frac{dG^*(t)}{dt} - \frac{dX(t)}{dt}G^*(t) \right] \\
&= -S_G(-S_G G_0^*) - \lim_{t \to 0} \left[G^*(t)(p_3[I(t) - I_b] - p_2 X(t)) \right] \\
&= S_G^2 G_0^* - G_0^* \lim_{t \to 0} \left[(p_3[I(t) - I_b] - p_2 X(t) \right]
\end{aligned}$$

$$= S_G^2 G_0^* - G_0^* p_3 \left[\lim_{t \to 0} I(t) - I_b \right].$$

As $t \to 0$, $I(t) \to I_0 = I_b$. Therefore, the second derivative does not provide any new information about the remaining parameters p_2, p_3. The third derivative gives

$$\begin{aligned}
y'''(t_0^+) &= -S_G \frac{d^2 G^*(0)}{dt^2} - X(0) \frac{d^2 G^*(0)}{dt^2} - 2 \frac{dX(0)}{dt} \frac{dG^*(0)}{dt} - \frac{d^2 X(0)}{dt^2} G^*(0) \\
&= -S_G \left(S_G G_0^* - p_3 G_0^* [I_0 - I_b] \right) - 2(p_3[I_0 - I_b])(-S_G G_0^*) - \frac{d^2 X(0)}{dt^2} G_0^* \\
&= -S_G^2 G_0^* - G_0^* \left(p_3 \frac{dI(0)}{dt} \right) \\
&= -S_G^2 G_0^* - G_0^* p_3 z'(0).
\end{aligned}$$

Hence,

$$p_3 = \frac{-S_G^2 G_0^* - y'''(0)}{G_0^* z'(0)}.$$

We conclude that p_3 is uniquely identifiable. Note that $G_0^* > 0$, and $z'(0) = I'(0) \neq 0$ since insulin should increase as an immediate response to the injected G_0^*.

From equation (3.22),

$$\begin{aligned}
y^{(4)}(t_0^+) &= -S_G \frac{d^3 G^*(t_0^+)}{dt^3} - \sum_{k=0}^{3} \binom{3}{0} \frac{d^{(3-k)} X(t_0^+)}{dt^{(3-k)}} \frac{d^k G^*(t_0^+)}{dt^k} \\
&= -S_G(-S_G^2 G_0^* - G_0^* p_3 z'(0)) - \frac{d^3 X(t_0^+)}{dt^3} G(t_0^+) - 3 \frac{d^2 X(t_0^+)}{dt^2} \frac{dG^*(t_0^+)}{dt} - \\
&\quad - 3 \frac{dX(t_0^+)}{dt} \frac{d^2 G^*(t_0^+)}{dt^2} - X(t_0^+) \frac{d^3 G^*(t_0^+)}{dt^3}.
\end{aligned}$$

In order to simplify this, we evaluate the limit as $t \to t_0^+$ for X and its derivatives as follows:

$$\begin{aligned}
X(t_0^+) &= \lim_{t \to 0} X(t) = 0 \\
\frac{dX(t_0^+)}{dt} &= \lim_{t \to 0} \frac{dX(t)}{dt} = \lim_{t \to 0} \left[p_3[I(t) - I_b] - p_2 X(t) \right] = p_3 \lim_{t \to 0} [I(t) - I_b] = 0 \\
\frac{d^2 X(t_0^+)}{dt^2} &= \lim_{t \to 0} \frac{d^2 X(t)}{dt^2} = \lim_{t \to 0} \left[p_3 \frac{dI(t)}{dt} - p_2 \frac{dX(t)}{dt} \right] = p_3 \lim_{t \to 0} \frac{dI(t)}{dt} = p_3 \frac{dI(0)}{dt} = p_3 z'(0)
\end{aligned}$$

$$\frac{d^3X(t_0^+)}{dt^3} = \lim_{t\to 0}\frac{d^3X(t)}{dt^3} = \lim_{t\to 0}\left[p_3\frac{d^2I(t)}{dt^2} - p_2\frac{d^2X(t)}{dt^2}\right] = p_3\frac{d^2I(0)}{dt^2} - p_2p_3z'(0) = p_3z''(0) - p_2p_3z'(0).$$

Plugging these into our equation gives

$$\begin{aligned} y^{(4)}(t_0^+) &= S_G^3G_0^* + S_GG_0^*p_3z'(0) - \big(p_3z''(0) - p_2p_3z'(0)\big)G_0^* - 3\big(p_3z'(0)\big)\big(-S_GG_0^*\big) \\ &= S_G^3G_0^* + S_GG_0^*p_3z'(0) - G_0^*p_3z''(0) + G_0^*p_2p_3z'(0) + 3S_GG_0^*p_3z'(0). \end{aligned}$$

Thus p_2 is uniquely identifiable, and is given by

$$p_2 = \frac{y^{(4)}(0) - S_G^3G_0^* - S_GG_0^*p_3z'(0) + G_0^*p_3z''(0) - 3S_GG_0^*p_3z'(0)}{G_0^*p_3z'(0)}.$$

Having established that the parameters S_G, p_2, p_3 are uniquely identifiable, we conclude that this model is uniquely identifiable.

For completeness, recall that $S_G = \dfrac{p_1}{V} = \dfrac{k_1^L + k_1^P}{V}$. We have shown that S_G is uniquely identifiable, but the parameters k_1^L, k_1^P, and V are not since the combination $\dfrac{k_1^L + k_1^P}{V}$ has infinitely many solutions for the parameters k_1^L, k_1^P, V. Therefore, as established at the beginning of this section, the original Minimal Model for labeled glucose dynamics is system unidentifiable.

3.3.2 UNLABELED MINIMAL MODEL

Recall the unlabeled glucose minimal model:

$$\left.\begin{aligned} \frac{dG(t)}{dt} &= \frac{-(k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t) + B_0}{V}, & G(0) = G_0 \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), & \hat{I}(0) = 0 \\ y(t) &= G(t). & \end{aligned}\right\} \tag{3.23}$$

The identifiability analysis of this model follows directly from the analysis above, except we note the additional term B_0 in the numerator, which is the extrapolated value for NHGP at zero glucose. We assume $G(t)$ and $I(t)$ have infinitely many derivatives with respect to time and the state components. Again applying the General Leibniz Rule (3.21) to $y(t)$, we obtain a similar result to equation (3.22):

$$
\begin{aligned}
y(t) &= G(t) \\
y'(t) &= -\frac{k_1}{V}G(t) - \frac{k_4}{V}\hat{I}(t)G(t) + \frac{B_0}{V} \\
y''(t) &= -\frac{k_1}{V}\frac{dG(t)}{dt} - \frac{k_4}{V}\left[\frac{d\hat{I}(t)}{dt}G(t) + \hat{I}(t)\frac{dG(t)}{dt}\right] \\
&\vdots
\end{aligned}
$$

$$
y^{(n)}(t) = -\frac{k_1}{V}\frac{d^{(n-1)}G(t)}{dt^{n-1}} - \frac{k_4}{V}\sum_{k=0}^{n-1}\binom{n-1}{k}\frac{d^{(n-1-k)}\hat{I}(t)}{dt^{(n-1-k)}}\frac{d^{(k)}G(t)}{dt^{(k)}}, \qquad n \geq 2. \tag{3.24}
$$

We will consider the identifiability about $t_0 = 0$. First, we have $y(0) = G(0) = G_0$. Hence, G_0 is a known value. Next,

$$
\begin{aligned}
\dot{y}(0) &= -\frac{k_1}{V}G(0) - \frac{k_4}{V}\hat{I}(0)G(0) + \frac{B_0}{V} \\
&= \frac{-k_1 G_0 + B_0}{V}
\end{aligned}
$$

which implies

$$
B_0 = V\dot{y}(0) + (k_1^L + k_1^P)G_0.
$$

Since $B_0, \dot{y}(0), G_0$ are known, this equation describes a linear relationship between V and $(k_1^L + k_1^P)$. Therefore, we conclude that this model is system unidentifiable.

Now we will consider the unlabeled minimal model with the parameter combinations defined in (3.6):

$$\left.\begin{aligned} \frac{dG(t)}{dt} &= -[S_G + X(t)]G(t) + S_G G_b, \qquad & G(0) = G_0 \\ \frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), & X(0) = 0 \\ y(t) &= G(t) \\ z(t) &= I(t). \end{aligned}\right\} \tag{3.25}$$

Again, we compute the derivatives of $y(t)$ using the Leibniz Rule to get

$$\begin{aligned} y(t) &= G(t) \\ y'(t) &= -S_G G(t) - X(t)G(t) + S_G G_b \\ y''(t) &= -S_G \frac{dG(t)}{dt} - \left[\frac{dX(t)}{dt} G(t) + X(t)\frac{dG(t)}{dt}\right] \\ &\vdots \end{aligned}$$

$$y^{(n)}(t) = -S_G \frac{d^{(n-1)}G(t)}{dt^{n-1}} - \sum_{k=0}^{n-1} \binom{n-1}{k} \frac{d^{(n-1-k)}X(t)}{dt^{(n-1-k)}} \frac{d^{(k)}G(t)}{dt^{(k)}}, \qquad n \geq 2. \tag{3.26}$$

Trivially, G_0 is a known value. The first derivative evaluated at t as $t \to t_0^+ = 0^+$ gives

$$\begin{aligned} y'(t_0^+) &= -S_G G(t_0^+) - X(t_0^+)G(t_0^+) + S_G G_b \\ &= \lim_{t \to 0^+} \big[- S_G G(t) - X(t)G(t) + S_G G_b \big] \\ &= -S_G \lim_{t \to 0^+} G(t) + S_G G_b \\ &= -S_G G_0 + S_G G_b \\ &= 0 \end{aligned}$$

since $G_0 = G_b$ for unlabeled fasting glucose. Thus, the first derivative gives no new information for parameter identifiability. Evaluating the second derivative as $t \to t_0^+$ gives

$$\begin{aligned} y''(t_0^+) &= \lim_{t \to 0^+} \left[-S_G \frac{dG(t)}{dt} - \left[\frac{dX(t)}{dt} G(t) + X(t)\frac{dG(t)}{dt}\right]\right] \\ &= -\lim_{t \to 0^+} \left[\frac{dX(t)}{dt} G(t) + X(t)\frac{dG(t)}{dt}\right] \end{aligned}$$

$$= -\lim_{t\to 0^+}\left[X(t)\frac{dG(t)}{dt}\right]$$
$$= 0.$$

Again, this gives no new information for parameter identifiability. However, evaluating the third derivative as $t \to t_0^+$ gives

$$\begin{aligned}
y'''(t_0^+) &= -S_G\frac{d^2G(t_0^+)}{dt^2} - \frac{d^2X(t_0^+)}{dt^2}G(t_0^+) - 2\frac{dX(t_0^+)}{dt}\frac{dG(t_0^+)}{dt} - X(t_0^+)\frac{d^2G(t_0^+)}{dt^2} \\
&= -\lim_{t\to 0^+}\left[\frac{d^2X(t)}{dt^2}G(t)\right] \\
&= -p_3z'(0)G_0.
\end{aligned}$$

Thus $p_3 = -\dfrac{y'''(0)}{G_0z'(0)}$. Whence, p_3 is uniquely identifiable. Finally, we will consider the fourth derivative as $t \to t_0^+$:

$$\begin{aligned}
y^{(4)}(t_0^+) &= \lim_{t\to 0^+}\left[-S_G\frac{d^3G(t)}{dt^3} - \frac{d^3X(t)}{dt^3}G(t) - 3\frac{d^2X(t)}{dt^2}\frac{dG(t)}{dt} - 3\frac{dX(t)}{dt}\frac{d^2G(t)}{dt^2} - X(t)\frac{d^3G(t)}{dt^3}\right] \\
&= -S_G(-p_3z'(0)G_0) - (p_3z''(0) - p_2p_3z'(0))G_0 \\
&= S_GG_0p_3z'(0) - G_0p_3z''(0) + G_0p_2p_3z'(0).
\end{aligned}$$

Therefore, $p_2 = \dfrac{y^{(4)}(0) - S_GG_0p_3z'(0) + G_0p_3z''(0)}{G_0p_3z'(0)}$ is uniquely identifiable. We conclude that this model is uniquely identifiable.

3.4 IDENTIFIABILITY ANALYSIS FOR THE ORAL MINIMAL MODEL

For the following sections, we will assume $G(t), G_{\text{meal}}(t)$ have infinitely many derivatives with respect to time and state components. In addition, we make several biological assumptions. For the fasting steady state, $\dfrac{dG(t)}{dt} = 0$. The assumption $G_0 = G_b$ persists in the oral minimal model for total glucose because glucose coming from the meal at $t = 0$ is 0, $G_{\text{meal}}(0) = 0$, and $Ra_{\text{meal}}(0) = 0$. In the postprandial state, glucose dynamics are no longer constant since glucose coming from the meal is changing and endogenous sources are changing.

3.4.1 TOTAL GLUCOSE ORAL MINIMAL MODEL

We will start with the original Oral Minimal Model for total glucose:

$$\left.\begin{aligned} \frac{dG(t)}{dt} &= \frac{Ra_{\text{meal}}(t) + B_0 - (k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t)}{V}, \quad && G(0) = G_b \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), && \hat{I}(0) = 0 \\ y(t) &= G(t) \end{aligned}\right\} \tag{3.27}$$

where

$$Ra_{\text{meal}}(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \le t \le t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}. \tag{3.28}$$

As in §3.3, we will use the Taylor Expansion Method about $t_0 = 0^+$. Since Ra_{meal} is not differentiable at the breakpoints, we will consider the interval $0 \le t \le t_1$. The parameter vector is $\vec{p} = [k_1^L, k_1^P, k_2, k_3, k_4^L, k_4^P, V, B_0, \alpha_0, \ldots, \alpha_n]^T$ and includes $n + 9$ parameters. Note that since $Ra_{\text{meal}}(0) = \alpha_0 = 0$, α_0 is uniquely identifiable, and it suffices to consider the other $n + 8$ parameters. First, we have

$$y(t_0^+) = G(t_0^+) = G(0) = G_b$$

so G_b is known. Next,

$$\begin{aligned} y'(t_0^+) = \frac{dG(t_0^+)}{dt} &= \lim_{t \to 0^+} \left[\frac{Ra_{\text{meal}}(t) + B_0 - (k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t)}{V} \right] \\ &= \frac{B_0}{V} + \frac{1}{V} \lim_{t \to 0^+} \left[Ra_{\text{meal}}(t) - (k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t) \right]. \\ &= \frac{B_0}{V} + \frac{1}{V} \lim_{t \to 0^+} \left[-(k_1^L + k_1^P)G(t) - (k_4^L + k_4^P)\hat{I}(t)G(t) \right] \\ &= \frac{1}{V} \left[B_0 - (k_1^L + k_1^P)G_b \right]. \end{aligned}$$

Assuming B_0 is known, we arrive at

$$B_0 = V y'(0) + (k_1^L + k_1^P)G_b.$$

Since $B_0, y'(0)$, and G_b are known, this defines a linear relationship between V and $(k_1^L + k_1^P)$. Thus, this model is system unidentifiable.

We will now consider the final oral minimal model for total glucose. The model is given by

$$\left.\begin{aligned} \frac{dG(t)}{dt} &= -[S_G + X(t)]G(t) + S_G G_b + \frac{Ra_{\text{meal}}(t)}{V}, \quad G(0) = G_b \\ \frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), \qquad X(0) = 0 \\ y(t) &= G(t) \\ z(t) &= I(t). \end{aligned}\right\} \tag{3.29}$$

We consider parameters $\vec{p} = [S_G, V, p_2, p_3, \alpha_1, \ldots, \alpha_n]^T$ (since $\alpha_0 = 0$ is known). To begin, we see that G_b is a known value. Next,

$$\begin{aligned} y'(t_0^+) = \frac{dG(t)}{dt} &= \lim_{t \to 0^+} \left[-[S_G + X(t)]G(t) + S_G G_b + \frac{Ra_{\text{meal}}(t)}{V} \right] \\ &= \lim_{t \to 0^+} \left[-X(t)G(t) + \frac{Ra_{\text{meal}}(t)}{V} \right] \\ &= 0 \end{aligned}$$

since $X(0) = 0$ and $Ra_{\text{meal}}(0) = 0$. We note that this is what we would expect based on the biological assumptions: $y(t) = G(t), y'(t) = G'(t) = 0$ for total glucose. Before we consider the second derivative, note that the first derivative of Ra_{meal} is

$$\frac{d}{dt} Ra_{\text{meal}}(t) = \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}.$$

Now, the second derivative gives

$$\begin{aligned} y''(t_0^+) &= \lim_{t \to 0^+} \left[-S_G \frac{dG(t)}{dt} - \frac{dX(t)}{dt} G(t) - X(t) \frac{dG(t)}{dt} + \frac{1}{V} \frac{d}{dt} Ra_{\text{meal}}(t) \right] \\ &= \lim_{t \to 0^+} \left[-\frac{dX(t)}{dt} G(t) + \frac{1}{V} \frac{d}{dt} Ra_{\text{meal}}(t) \right] \\ &= \frac{1}{V} \frac{\alpha_1 - \alpha_0}{t_1 - t_0} - \lim_{t \to 0^+} \frac{dX(t)}{dt} G(t) \\ &= \frac{1}{V} \frac{\alpha_1}{t_1} \end{aligned}$$

since $\dfrac{dX(t_0^+)}{dt} = 0$ (see §3.3.1). This gives

$$\frac{\alpha_1}{V} = t_1 y''(0).$$

Therefore, the combination $\dfrac{\alpha_1}{V}$ is uniquely identifiable, but the parameters α_1 and V are not. Hence, this model is <u>system unidentifiable</u>.

Even though the identifiability of the system is not changed, we note that decreasing the number of parameters from $n+8$ to $n+4$ while still maintaining biological significance is desirable. Reducing the parameter space helps with potential computational limitations and can help show a potential method for overcoming identifiability issues.

Specifically, there is an approach to overcome this identifiability issue. If we fix V to be the average distribution volume for the subject population, then α_1 becomes uniquely identifiable, as well as all other parameters. At this point, we have shown α_0 and α_1. A similar result to §4.2 is produced when we consider $t_i, i = 2, \ldots, n$. We gain that $\alpha_2, \ldots, \alpha_n$ is uniquely identifiable, as well as S_G. In this case, the model would be <u>system identifiable</u>. This implies a numerical approach for determining p_2, p_3, V to calculate S_I.

3.4.2 MEAL GLUCOSE ORAL MINIMAL MODEL

Finally, we will consider the identifiability of the Oral Minimal Model for glucose coming from the meal (exogenous glucose), denoted G_{meal}. This model is given by

$$\left.\begin{aligned} \frac{dG_{\text{meal}}(t)}{dt} &= \frac{Ra_{\text{meal}}(t) - (k_1^L + k_1^P)G_{\text{meal}}(t) - (k_4^L + k_4^P)\hat{I}(t)G_{\text{meal}}(t)}{V}, \quad & G_{\text{meal}}(0) = 0 \\ \frac{d\hat{I}(t)}{dt} &= k_2[I(t) - I_b] - k_3\hat{I}(t), & \hat{I}(0) = 0 \\ y(t) &= G_{\text{meal}}(t) \\ z(t) &= I(t), \end{aligned}\right\} \tag{3.30}$$

with

$$Ra_{\text{meal}}(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \le t \le t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}. \tag{3.31}$$

First, we have

$$y(t) = G(t) \implies y(0) = G_{\text{meal}}(0) = 0.$$

The first derivative gives

$$\begin{aligned}
y'(t) = \frac{dG_{\text{meal}}(t)}{dt} &= \lim_{t \to 0^+} \left[\frac{Ra_{\text{meal}}(t) - (k_1^L + k_1^P)G_{\text{meal}}(t) - (k_4^L + k_4^P)\hat{I}(t)G_{\text{meal}}(t)}{V} \right] \\
&= - \lim_{t \to 0^+} \frac{(k_1^L + k_1^P)G_{\text{meal}}(t)}{V} \\
&= 0.
\end{aligned}$$

As this gives no new information for parameter identifiability, we consider the second derivative:

$$\begin{aligned}
y''(t_0^+) &= \frac{1}{V} \lim_{t \to 0^+} \left[\frac{d}{dt} Ra_{\text{meal}}(t) - (k_1^L + k_1^P) \frac{dG_{\text{meal}}(t)}{dt} - (k_4^L + k_4^P) \left(\frac{d\hat{I}(t)}{dt} G_{\text{meal}}(t) + \hat{I}(t) \frac{dG_{\text{meal}}(t)}{dt} \right) \right] \\
&= \frac{1}{V} \lim_{t \to 0^+} \left[\frac{\alpha_1 - \alpha_0}{t_1 - t_0} - (k_4^L + k_4^P) \left(\frac{d\hat{I}(t)}{dt} G_{\text{meal}}(t) \right) \right] \\
&= \frac{1}{V} \frac{\alpha_1 - \alpha_0}{t_1 - t_0} \\
&= \frac{1}{V} \frac{\alpha_1}{t_1}
\end{aligned}$$

Similar to the result in the previous section, we have $\frac{\alpha_1}{V} = t_1 y''(0)$. Thus, the combination $\frac{\alpha_1}{V}$ is identifiable but the parameters α_1 and V are nonidentifiable. Therefore, this model is system unidentifiable.

Finally, we will consider the final form of the oral minimal model for glucose coming from the meal, given by

$$\begin{cases}
\dfrac{dG_{\text{meal}}(t)}{dt} = -[S_G^* + X^*(t)]G_{\text{meal}}(t) + \dfrac{Ra_{\text{meal}}(t)}{V^*}, & G_{\text{meal}}(0) = 0 \\
\dfrac{dX^*(t)}{dt} = p_3^*[I(t) - I_b] - p_2^* X^*(t), & X^*(0) = 0 \\
y(t) = G_{\text{meal}}(t) \\
z(t) = I(t)
\end{cases} \tag{3.32}$$

with

$$Ra_{\text{meal}}(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \le t \le t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}. \tag{3.33}$$

Note the following:

$$\begin{aligned} y(t) &= G_{\text{meal}}(t) \\ y'(t) &= -[S_G^* + X^*(t)]G_{\text{meal}}(t) + \frac{Ra_{\text{meal}}(t)}{V^*} \\ y''(t) &= -S_G^* \frac{dG_{\text{meal}}(t)}{dt} - \frac{dX^*(t)}{dt} G_{\text{meal}}(t) - X^*(t) \frac{dG_{\text{meal}}(t)}{dt} + \frac{1}{V^*} \frac{d}{dt} Ra_{\text{meal}}(t) \\ &\vdots \\ y^{(n)}(t) &= -S_G^* \frac{d^{(n-1)} G_{\text{meal}}(t)}{dt^{(n-1)}} + \frac{1}{V^*} \frac{d^{(n-1)}}{dt^{(n-1)}} Ra_{\text{meal}}(t) - \sum_{k=0}^{n-1} \binom{n-1}{k} \frac{d^{(n-1-k)} X^*(t)}{dt^{(n-1-k)}} \frac{d^{(k)} G_{\text{meal}}(t)}{dt^{(k)}}. \end{aligned}$$

Since $Ra_{\text{meal}}(t)$ varies over different time intervals, the α_i parameters present in the system depend on the time interval of consideration. We begin by considering the system at t_0^+ which corresponds to the interval $t_0 = 0 \le t \le t_1$, with $t \to t_0^+$. In this case, we have the following additional information:

$$Ra_{\text{meal}}(t_0^+) = \lim_{t \to t_0^+} \left[\alpha_0 + \frac{\alpha_1 - \alpha_0}{t_1 - t_0}(t - t_0) \right] = \alpha_0$$

and

$$\begin{aligned} Ra_{\text{meal}}(0) = 0 &\implies \alpha_0 = 0, \\ \frac{d}{dt} Ra_{\text{meal}}(t_0^+) &= \lim_{t \to t_0^+} \left[\frac{\alpha_1 - \alpha_0}{t_1 - t_0} \right] = \frac{\alpha_1}{t_1} \end{aligned}$$

since $\alpha_0 = 0, t_0 = 0$. In addition, evaluating $X^*(t)$ and its derivatives as $t \to t_0^+$ gives the following:

$$\begin{aligned} X^*(t_0^+) &= \lim_{t \to t_0^+} X^*(t) = 0 \\ \frac{dX^*(t_0^+)}{dt} &= \lim_{t \to t_0^+} [p_3^*[I(t) - I_b] - p_2^* X^*(t)] = 0 \\ \frac{d^2 X^*(t_0^+)}{dt^2} &= \lim_{t \to t_0^+} \left[p_3^* \frac{dI(t)}{dt} - p_2^* \frac{dX^*(t)}{dt} \right] = p_3^* z'(0) \end{aligned}$$

$$\vdots$$

$$\frac{d^{(i)}X^*(t_0^+)}{dt^{(i)}} = p_3^* \sum_{j=1}^{i-1} (-1)^{j-1}(p_2^*)^{j-1} z^{(i-j)}(0), \quad \forall i \in \mathbb{N}.$$

Now we will apply the method for t_0^+.

$$\begin{aligned}
y(t_0^+) =& G_{\text{meal}}(t_0^+) = 0 \\
y'(t_0^+) =& -[S_G^* + X^*(t_0^+)]G_{\text{meal}}(t_0^+) + \frac{Ra_{\text{meal}}(t_0^+)}{V^*} \\
=& -[S_G^* + 0] \cdot 0 + \frac{0}{V^*} \\
=& 0 \\
y''(t_0^+) =& -S_G^* \frac{dG_{\text{meal}}(t_0^+)}{dt} - \frac{dX^*(t_0^+)}{dt} G_{\text{meal}}(t_0^+) - X^*(t_0^+) \frac{dG_{\text{meal}}(t_0^+)}{dt} + \frac{1}{V^*} \frac{d}{dt} Ra_{\text{meal}}(t_0^+) \\
=& -S_G^* \cdot 0 - \frac{dX^*(t_0^+)}{dt} \cdot 0 - 0 \cdot \frac{dG_{\text{meal}}(t_0^+)}{dt} + \frac{1}{V^*} \frac{\alpha_1}{t_1} \\
=& \frac{1}{V^*} \frac{\alpha_1}{t_1} \\
y'''(t_0^+) =& -S_G^* \frac{d^2 G_{\text{meal}}(t_0^+)}{dt^2} - \frac{d^2 X^*(t_0^+)}{dt^2} G_{\text{meal}}(t_0^+) - 2 \frac{dX^*(t_0^+)}{dt} \frac{dG_{\text{meal}}(t_0^+)}{dt} - X^*(t_0^+) \frac{d^2 G_{\text{meal}}(t_0^+)}{dt^2} + \\
& + \frac{1}{V^*} \frac{d^2}{dt^2} Ra_{\text{meal}}(t_0^+) \\
=& -S_G^* y''(t_0^+) - \frac{d^2 X^*(t_0^+)}{dt^2} \cdot 0 - 2 \cdot 0 \cdot \frac{dG_{\text{meal}}(t_0^+)}{dt} - 0 \cdot \frac{d^2 G_{\text{meal}}(t_0^+)}{dt^2} + \frac{1}{V^*} \cdot 0 \\
=& -S_G^* y''(t_0^+) \\
y^{(4)}(t_0^+) =& -S_G^* \frac{d^3 G_{\text{meal}}(t_0^+)}{dt^3} - \frac{d^3 X^*(t_0^+)}{dt^3} G_{\text{meal}}(t_0^+) - 3 \frac{d^2 X^*(t_0^+)}{dt^2} \frac{dG_{\text{meal}}(t_0^+)}{dt} - 3 \frac{dX^*(t_0^+)}{dt} \frac{d^2 G_{\text{meal}}(t_0^+)}{dt^2} - \\
& - X^*(t_0^+) \frac{d^3 G_{\text{meal}}(t_0^+)}{dt^3} + \frac{1}{V^*} \frac{d^3}{dt^3} Ra_{\text{meal}}(t_0^+) \\
=& -S_G^* y'''(t_0^+) - \frac{d^3 X^*(t_0^+)}{dt^3} \cdot 0 - 3 \frac{d^2 X^*(t_0^+)}{dt^2} \cdot 0 - 3 \cdot 0 \cdot \frac{d^2 G_{\text{meal}}(t_0^+)}{dt^2} - 0 \cdot \frac{d^3 G_{\text{meal}}(t_0^+)}{dt^3} + \frac{1}{V^*} \cdot 0 \\
=& -S_G^* y'''(t_0^+)
\end{aligned}$$

For $k \geq 5$, we may express the k^{th} derivative of y as $t \to t_0^+$ as follows:

$$y^{(k)}(t_0^+) = -S_G^* y^{(k-1)}(t_0^+) - \sum_{i=2}^{k-3} \binom{k-1}{i} \frac{d^i X^*(t_0^+)}{dt^i} y^{(k-1-i)}(t_0^+). \tag{3.34}$$

We use this expression to obtain the fifth and sixth derivatives of y as $t \to t_0^+$:

$$
\begin{aligned}
y^{(5)}(t_0^+) =& S_G^* y^{(4)}(t_0^+) - 6\Big[p_3^* z'(0)\Big] y''(t_0^+) \\
y^{(6)}(t_0^+) =& - S_G^* y^{(5)}(t_0^+) - 10\Big[p_3^* z''(0) - p_2^* p_3^* z'(0)\Big] y''(t_0^+) - 10\Big[p_3^* z'(0)\Big] y'''(t_0^+).
\end{aligned}
$$

To recall, on this interval the unknowns are $\vec{p} = [S_G^*, p_2^*, p_3^*, V^*, \alpha_1]^T$. Summarizing the equations above, we can represent the unknown parameters in terms of the following derivatives which are assumed to be known:

$$y''(t_0^+) = \frac{1}{V^*} \frac{\alpha_1}{t_1} \tag{3.35}$$

$$y'''(t_0^+) = -S_G^* y''(t_0^+) \tag{3.36}$$

$$y^{(5)}(t_0^+) = S_G^* y^{(4)}(t_0^+) - 6\Big[p_3^* z'(0)\Big] y''(t_0^+) \tag{3.37}$$

$$y^{(6)}(t_0^+) = -S_G^* y^{(5)}(t_0^+) - 10\Big[p_3^* z''(0) - p_2^* p_3^* z'(0)\Big] y''(t_0^+) - 10\Big[p_3^* z'(0)\Big] y'''(t_0^+). \tag{3.38}$$

Starting with (3.36), we see that

$$S_G^* = -\frac{y'''(t_0^+)}{y''(t_0^+)} \tag{3.39}$$

is uniquely identifiable. Next, consider (3.37). This gives

$$p_3^* = \frac{S_G^* y^{(4)}(t_0^+) - y^{(5)}(t_0^+)}{6z'(0) y''(t_0^+)} \tag{3.40}$$

is uniquely identifiable. Now, when we consider (3.38), we get

$$p_2^* = \frac{y^{(6)}(t_0^+) + S_G^* y^{(5)}(t_0^+) + 10 p_3^* z''(0) y''(t_0^+) + 10 p_3^* z'(0) y'''(t_0^+)}{10 p_3^* z'(0) y''(t_0^+)} \tag{3.41}$$

is also uniquely identifiable. It remains to consider the identifiabiliity of V^* and α_1. Separating knowns (right-hand-side) and unknowns (left-hand-side), we obtain $\frac{\alpha_1}{V^*} = t_1 y''(0)$. Thus the parameter combination $\frac{\alpha_1}{V^*}$ is uniquely identifiable but the individual parameters α_1 and V^* are not. Since both α_1 and V^* drop out of the y-derivatives after the second derivative, we can not obtain any additional information about these parameters from higher derivatives. Therefore, this entire model is system unidentifiable. In order to overcome this, one must either fix α_1 or V^*. These two cases are represented by RM (fixing α_1 using Steele's Ra) and FM (fixing V^* from results from

RM).

Now we may consider t_1^+. This corresponds to $t_1 \le t \le t_2$, $t \to t_1^+$. As $X^*(t_1^+)$ is unknown, we use caution using this approach. We have

$$\begin{aligned}
y(t_1^+) &= \lim_{t\to t_1^+} G_{\text{meal}}(t_1^+) \\
&= G_{\text{meal}}(t_1) \\
y'(t_1^+) &= \lim_{t\to t_1^+} \left[-[S_G^* + X^*(t)]G_{\text{meal}}(t) + \frac{Ra_{\text{meal}}(t)}{V^*} \right] \\
&= -[S_G^* + X^*(t_1^+)]y(t_1^+) + \frac{\alpha_2}{V^*}.
\end{aligned}$$

As we can see, not knowing $X^*(t_1^+)$ will force us to slightly modify the approach. To solve for $S^*(t_1^+)$ in terms of known quantities, we take the first, second, and third derivatives of the entire system:

$$\begin{cases}
\dfrac{dG_{\text{meal}}(t_1^+)}{dt} &= -[S_G^* + X^*(t_1^+)]G_{\text{meal}}(t_1^+) + \dfrac{Ra_{\text{meal}}(t_1^+)}{V^*} \\
&= -[S_G^* + X^*(t_1^+)]G_{\text{meal}}(t_1^+) + \dfrac{\alpha_1}{V^*} \\
\dfrac{dX^*(t_1^+)}{dt} &= p_3^*[I(t_1^+) - I_b] - p_2^* X^*(t_1^+)
\end{cases} \tag{3.42}$$

$$\begin{cases}
\dfrac{d^2G_{\text{meal}}(t_1^+)}{dt^2} &= - S_G^* \dfrac{dG_{\text{meal}}(t_1^+)}{dt} - \dfrac{dX^*(t_1^+)}{dt} G_{\text{meal}}(t_1^+) - X^*(t_1^+)\dfrac{dG_{\text{meal}}(t_1^+)}{dt} + \\
&\quad + \dfrac{1}{V^*}\dfrac{d}{dt} Ra_{\text{meal}}(t_1^+) \\
&= - [S_G^* + X^*(t_1^+)]\dfrac{dG_{\text{meal}}(t_1^+)}{dt} - \dfrac{dX^*(t_1^+)}{dt} G_{\text{meal}}(t_1^+) + \\
&\quad + \dfrac{1}{V^*}\dfrac{\alpha_2 - \alpha_1}{t_2 - t_1} \\
\dfrac{d^2X^*(t_1^+)}{dt^2} &= p_3^* \dfrac{dI(t_1^+)}{dt} - p_2^* \dfrac{dX^*(t_1^+)}{dt}
\end{cases} \tag{3.43}$$

and

$$
\begin{cases}
\dfrac{d^3G_{\text{meal}}(t_1^+)}{dt^3} &= -\, S_G^* \dfrac{d^2G_{\text{meal}}(t_1^+)}{dt^2} - \dfrac{d^2X^*(t_1^+)}{dt^2} G_{\text{meal}}(t_1^+) - 2\dfrac{dX^*(t_1^+)}{dt}\dfrac{dG_{\text{meal}}(t_1^+)}{dt} - \\
&\quad - X^*(t_1^+)\dfrac{d^2G_{\text{meal}}(t_1^+)}{dt^2} + \dfrac{1}{V^*}\dfrac{d^2}{dt^2} Ra_{\text{meal}}(t_1^+) \\
&= -\,[S_G^* + X^*(t_1^+)]\dfrac{d^2G_{\text{meal}}(t_1^+)}{dt^2} - \\
&\quad - \dfrac{d^2X^*(t_1^+)}{dt^2} G_{\text{meal}}(t_1^+) - 2\dfrac{dX^*(t_1^+)}{dt}\dfrac{dG_{\text{meal}}(t_1^+)}{dt} \\
\dfrac{d^3X^*(t_1^+)}{dt^3} &= p_3^* \dfrac{d^2I(t_1^+)}{dt^2} - p_2^* \dfrac{d^2X^*(t_1^+)}{dt^2}.
\end{cases}
\tag{3.44}
$$

Substituting information from the second equations in (3.42) and (3.43) into the first equation in (3.44), we obtain the following:

$$
\begin{aligned}
\frac{d^3G_{\text{meal}}(t_1^+)}{dt^3} &= -\,[S_G^* + X^*(t_1^+)]\frac{d^2G_{\text{meal}}(t_1^+)}{dt^2} - \frac{d^2X^*(t_1^+)}{dt^2} G_{\text{meal}}(t_1^+) - 2\frac{dX^*(t_1^+)}{dt}\frac{dG_{\text{meal}}(t_1^+)}{dt} \\
&= -\,[S_G^* + X^*(t_1^+)]y''(t_1^+) - \left[p_3^*\frac{dI(t_1^+)}{dt} - p_2^*\frac{dX^*(t_1^+)}{dt}\right] y(t_1^+) - \\
&\quad - 2\Big[p_3^*[I(t_1^+) - I_b] - p_2^* X^*(t_1^+)\Big] y'(t_1^+) \\
&= -\,[S_G^* + X^*(t_1^+)]y''(t_1^+) - \big[p_3^* z'(t_1^+) - p_2^*\big(p_3^*[I(t_1^+) - I_b] - p_2^* X^*(t_1^+)\big)\big]\, y(t_1^+) - \\
&\quad - 2\Big[p_3^*[I(t_1^+) - I_b] - p_2^* X^*(t_1^+)\Big] y'(t_1^+).
\end{aligned}
$$

Since $y'''(t_1^+) = \dfrac{d^3G_{\text{meal}}(t_1^+)}{dt^3}$, we have

$$
\begin{aligned}
y'''(t_1^+) &= -\, S_G^* y''(t_1^+) - X^*(t_1^+)y''(t_1^+) - p_3^* z'(t_1^+)y(t_1^+) + p_2^* y(t_1^+)\big(p_3^*[z(t_1^+) - I_b] - p_2^* X^*(t_1^+)\big) - \\
&\quad - 2p_3^* y'(t_1^+)[z(t_1^+) - I_b] + 2p_2^* y'(t_1^+)X^*(t_1^+)
\end{aligned}
$$

$$
\begin{aligned}
&= -\, S_G^* y''(t_1^+) - X^*(t_1^+)y''(t_1^+) - p_3^* z'(t_1^+)y(t_1^+) + p_2^* p_3^* y(t_1^+)[z(t_1^+) - I_b] - (p_2^*)^2 y(t_1^+)X^*(t_1^+) - \\
&\quad - 2p_3^* y'(t_1^+)[z(t_1^+) - I_b] + 2p_2^* y'(t_1^+)X^*(t_1^+)
\end{aligned}
$$

$$
\begin{aligned}
&= [2p_2^* y'(t_1^+) - (p_2^*)^2 y(t_1^+) - y''(t_1^+)]X^*(t_1^+) - \\
&\quad - S_G^* y''(t_1^+) - p_3^* z'(t_1^+)y(t_1^+) + p_2^* p_3^* y(t_1^+)[z(t_1^+) - I_b] - 2p_3^* y'(t_1^+)[z(t_1^+) - I_b].
\end{aligned}
$$

Hence,

$$X^*(t_1^+) = \frac{y'''(t_1^+) + S_G^* y''(t_1^+) + p_3^* z'(t_1^+) y(t_1^+) - p_2^* p_3^* y(t_1^+)[z(t_1^+) - I_b] + 2p_3^* y'(t_1^+)[z(t_1^+) - I_b]}{2p_2^* y'(t_1^+) - (p_2^*)^2 y(t_1^+) - y''(t_1^+)}. \tag{3.45}$$

We have shown that $X^*(t_1^+)$ can be written as a function of G, I and their derivatives as well as S_G^*, p_2^* and p_3^*, which are all known quantities so $X^*(t_1^+)$ is known by the above equation. We will now substitute this expression into the second equation in (3.42):

$$\begin{aligned}
\frac{dX^*(t_1^+)}{dt} =& p_3^*[I(t_1^+) - I_b] - p_2^* X^*(t_1^+) \\
=& p_3^*[z(t_1^+) - I_b] - \\
& - p_2^* \frac{y'''(t_1^+) + S_G^* y''(t_1^+) + p_3^* z'(t_1^+) y(t_1^+) - p_2^* p_3^* y(t_1^+)[z(t_1^+) - I_b] + 2p_3^* y'(t_1^+)[z(t_1^+) - I_b]}{2p_2^* y'(t_1^+) - (p_2^*)^2 y(t_1^+) - y''(t_1^+)} \\
=& p_3^*[z(t_1^+) - I_b] - \\
& - \frac{y'''(t_1^+) + S_G^* y''(t_1^+) + p_3^* z'(t_1^+) y(t_1^+) - p_2^* p_3^* y(t_1^+)[z(t_1^+) - I_b] + 2p_3^* y'(t_1^+)[z(t_1^+) - I_b]}{2y'(t_1^+) - p_2^* y(t_1^+) - y''(t_1^+)}.
\end{aligned}$$

This gives us a new equation for $\dfrac{dX^*(t_1^+)}{dt}$. Using this, we will finally consider the first equation in (3.43):

$$\begin{aligned}
\frac{d^2 G_{\text{meal}}(t_1^+)}{dt^2} =& - S_G^* \frac{dG_{\text{meal}}(t_1^+)}{dt} - \frac{dX^*(t_1^+)}{dt} G_{\text{meal}}(t_1^+) - X^*(t_1^+) \frac{dG_{\text{meal}}(t_1^+)}{dt} + \frac{1}{V^*} \frac{d}{dt} Ra_{\text{meal}}(t_1^+) \\
=& - S_G^* y'(t_1^+) - \frac{dX^*(t_1^+)}{dt} y(t_1^+) - X^*(t_1^+) y'(t_1^+) + \frac{1}{V^*} \frac{\alpha_2 - \alpha_1}{t_2 - t_1}.
\end{aligned}$$

Thus, we have

$$\frac{\alpha_2 - \alpha_1}{V^*} = (t_2 - t_1) \left(y''(t_1^+) + S_G^* y'(t_1^+) + \frac{dX^*(t_1^+)}{dt} y(t_1^+) + X^*(t_1^+) y'(t_1^+) \right). \tag{3.46}$$

As $\frac{\alpha_1}{V^*}$ was determined from the analysis for t_0^+, we may substitute this information to obtain

$$\frac{\alpha_2}{V^*} = (t_2 - t_1)\left(y''(t_1^+) + S_G^* y'(t_1^+) + \frac{dX^*(t_1^+)}{dt} y(t_1^+) + X^*(t_1^+) y'(t_1^+)\right) + t_1 y''(t_0^+). \tag{3.47}$$

The right hand side of (3.47) is known since S_G^*, p_2^*, p_3^* are uniquely identifiable from considering t_0^+, and $y(t_1^+), y'(t_1^+), \ldots, z(t_1^+), z'(t_1^+), \ldots$ are known by definition, and we know $X^*(t_1^+)$ and $\frac{dX^*(t_1^+)}{dt}$. The combination $\frac{\alpha_2}{V^*}$ is uniquely identifiable but α_2 and V^* are not uniquely identifiable. As in the analysis for t_0^+, additional derivatives do not give further information on α_1 and V^*. Again, we see that this model is system unidentifiable.

Lastly, we will consider $t_i, i = 2, \ldots, n-1$. Here, $t_i \le t \le t_{i+1}$, $t \to t_i^+$. We will use a similar method to t_1^+. We have

$$\begin{cases} \dfrac{dG_{\text{meal}}(t_i^+)}{dt} = -[S_G^* + X^*(t_i^+)]G_{\text{meal}}(t_i^+) + \dfrac{\alpha_i}{V^*} \\ \dfrac{dX^*(t_i^+)}{dt} = p_3^*[I(t_i^+) - I_b] - p_2^* X^*(t_i^+) \end{cases} \tag{3.48}$$

$$\begin{cases} \dfrac{d^2G_{\text{meal}}(t_i^+)}{dt^2} = -[S_G^* + X^*(t_i^+)]\dfrac{dG_{\text{meal}}(t_i^+)}{dt} - \dfrac{dX^*(t_i^+)}{dt}G_{\text{meal}}(t_i^+) + \dfrac{1}{V^*}\dfrac{d}{dt}Ra_{\text{meal}}(t_i^+) \\ \dfrac{d^2X^*(t_i^+)}{dt^2} = p_3^*\dfrac{dI(t_i^+)}{dt} - p_2^*\dfrac{dX^*(t_i^+)}{dt} \end{cases} \tag{3.49}$$

and

$$\begin{cases} \dfrac{d^3G_{\text{meal}}(t_i^+)}{dt^3} = -\left[S_G^* + X^*(t_i^+)\right]\dfrac{d^2G_{\text{meal}}(t_i^+)}{dt^2} - \dfrac{d^2X^*(t_i^+)}{dt^2}G_{\text{meal}}(t_i^+) - 2\dfrac{dX^*(t_i^+)}{dt}\dfrac{dG_{\text{meal}}(t_i^+)}{dt} \\ \dfrac{d^3X^*(t_i^+)}{dt^3} = p_3^*\dfrac{d^2I(t_i^+)}{dt^2} - p_2^*\dfrac{d^2X^*(t_i^+)}{dt^2}. \end{cases} \tag{3.50}$$

Just like the previous case, we start with the first equation in (3.50) and substitute information from the second equation in (3.49) and the second equation in (3.48) to obtain

$$\frac{d^3G_{\text{meal}}(t_i^+)}{dt^3} = -\left[S_G^* + X^*(t_i^+)\right]y''(t_i^+) - \left[p_3^* z'(t_i^+) - p_2^*\big(p_3^*[I(t_i^+) - I_b] - p_2^* X^*(t_i^+)\big)\right] y(t_i^+) -$$

$$- 2\Big[p_3^*[I(t_i^+) - I_b] - p_2^* X^*(t_i^+)\Big] y'(t_i^+).$$

We get a similar expression for $X(t_i^+)$:

$$X^*(t_i^+) = \frac{y'''(t_i^+) + S_G^* y''(t_i^+) + p_3^* z'(t_i^+) y(t_i^+) - p_2^* p_3^* y(t_i^+)[z(t_i^+) - I_b] + 2p_3^* y'(t_i^+)[z(t_i^+) - I_b]}{2p_2^* y'(t_i^+) - (p_2^*)^2 y(t_i^+) - y''(t_i^+)}. \tag{3.51}$$

From the first equation in (3.49),

$$\begin{aligned}
\frac{d^2 G_{\text{meal}}(t_i^+)}{dt^2} &= -[S_G^* + X^*(t_i^+)] \frac{dG_{\text{meal}}(t_i^+)}{dt} - \frac{dX^*(t_i^+)}{dt} G_{\text{meal}}(t_i^+) + \frac{1}{V^*} \frac{d}{dt} Ra_{\text{meal}}(t_i^+) \\
&= -S_G^* y'(t_i^+) - X^*(t_i^+) y'(t_i^+) - \frac{dX^*(t_i^+)}{dt} y(t_i^+) + \frac{\alpha_i - \alpha_{i-1}}{V^*(t_i - t_{i-1})}.
\end{aligned}$$

Hence,

$$\frac{\alpha_i}{V^*} = (t_i - t_{i-1})(y''(t_i^+) + S_G^* y'(t_i^+) + X^*(t_i^+) y'(t_i^+) + \frac{dX^*(t_i^+)}{dt} y(t_i^+)) + \frac{\alpha_{i-1}}{V^*}. \tag{3.52}$$

Again, we see that $\frac{\alpha_i}{V^*}$ is uniquely identifiable but α_i and V^* are not uniquely identifiable. Therefore, this model is system unidentifiable. In order to overcome this unidentifiability, one must specify either α_i for $i = 0, \ldots, n$ or V^*. These two cases are represented by the Reference Model (RM) and Final Model (FM), respectively, as described in the next section.

3.5 PARAMETER ESTIMATION

This section will discuss the numerical implementation of OMM*, starting with the algorithm, discuss a test to show the code works properly, and end with results for individual subjects. This section focuses on the following systems for total glucose (3.53) and exogenous glucose (3.54):

$$\left.\begin{aligned}
\frac{dG(t)}{dt} &= -[S_G + X(t)]G(t) + S_G G_b + \frac{Ra_{\text{meal}}(t)}{V}, & G(0) &= G_b \\
\frac{dX(t)}{dt} &= p_3[I(t) - I_b] - p_2 X(t), & X(0) &= 0
\end{aligned}\right\} \tag{3.53}$$

$$\left.\begin{aligned}
\frac{dG_{\text{meal}}(t)}{dt} &= -[S_G^* + X^*(t)]G_{\text{meal}}(t) + \frac{Ra_{\text{meal}}(t)}{V^*}, & G_{\text{meal}}(0) &= 0 \\
\frac{dX^*(t)}{dt} &= p_3^*[I(t) - I_b] - p_2^* X^*(t), & X^*(0) &= 0
\end{aligned}\right\} \tag{3.54}$$

with

$$Ra_{\text{meal}}(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \le t \le t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}. \tag{3.55}$$

3.5.1 ALGORITHM

Due to structural identifiability issues, we will use a two phase code. The first phase, called the Reference Model (RM), will used Steele's non-steady-state calculation for Ra_{meal} so $\alpha_i, i = 0, \ldots, n$ is known. The total glucose system and meal glucose system can be numerically identified separately and we will refer to these implementations as RM for total glucose and RM* for meal glucose. RM and RM* will estimate S_G, V and S_G^*, V^*, respectively, for use in the Final Model (FM). The second phase, FM, will use information obtained from RM and RM* to estimate p_2, p_3, p_2^*, p_3^*, and $\alpha_i, i = 0, \ldots, n$. The algorithm is as follows:

Load data

Set appropriate initial value for parameters

RM: S_G, p_2, p_3, V; ($\vec{\alpha}$ known)

RM: S_G^*, p_2^*, p_3^*, V^*; ($\vec{\alpha}$ known)*

FM: p_2, p_3, p_2^*, p_3^*, $\vec{\alpha}$; (S_G, S_G^*, V^*, V known)

Use fmincon to find the minimum of the error function subject to defined constraints

fmincon uses ode45 with given input parameters to calculate error

The error function uses the 2-norm: $||data - numerical\ result||_2$

Constraints:

RM: nonnegative parameters

RM: nonnegative parameters*

FM: nonnegative parameters, additional constraint on Ra

Save parameters that minimize the error function

Use ode45 to generate solution with this parameter set and plot the results

The built-in MATLAB minimizer **fmincon** minimizes the objective function subject to user-specified constraints. We use the minimizer **fmincon** because we want to ensure that our parameters are nonnegative, however fmincon does not need to explore the entire parameter space

$[0, \infty)$. Computationally, it can be expensive to search the entire space, so we can safely restrict the search space to (0,10), since *a priori* parameter values are much less than 10 for all parameters in RM, RM*, FM.

In FM, we additionally constrain Ra_{meal} with

$$\int_0^T Ra_{\text{meal}}(t)dt = fD \tag{3.56}$$

where f is the fraction absorbed, D is the ingested meal glucose dose, and T is the time required for glucose concentration to return to its basal value following the oral glucose challenge. As the subjects in our study did not return to basal values, we relax this constraint to

$$\int_0^{T_{\text{final}}} Ra_{\text{meal}}(t)dt \leq fD, \tag{3.57}$$

where $T \geq T_{\text{final}} = 240$ min. is the end time of our protocol.

3.5.2 CODE VALIDATION USING PUBLISHED DATA

In order to validate the numerical implementation, we tested the code with data from [11]. This source considers 88 normal subjects and includes plots of total glucose, meal glucose, insulin, Steele's Ra_{meal}, and the computational result for Ra_{meal} for the average values for all the subjects. We used these plots to estimate values for the data. We implemented RM, RM*, and FM as described above, and we compared the resulting parameter estimates to the published parameter values for RM* and FM. Since, the total ingested dose, D, was not given in [11], we estimated this constraint in our numerical implementation. The estimated data is summarized in Table 1.

In Table 2, the parameter values from [11] are given. The parameters p_3 and p_3^* were not given in [11], but we calculated these values from the other parameters.

The results of our numerical implementation of RM and RM* are presented in Figure 3.3 and Figure 3.4, respectively. In [11], the authors do not provide RM parameter values, so we rely on visual justification. Based on visual inspection, the numerical implementation appears to behave as desired. For RM*, we have agreement with S_G^* but our V^* is slightly low compared to reported

Table 1: Estimates for Cobelli Data

Time (mins)	Total Glucose Concentration (mg/dL)	Meal Glucose Concentration (mg/dL)	Insulin Concentration (μ U/mL)	Steele's Ra_{meal} mg$\cdot$ kg^{-1} $\cdot$ min^{-1}
0	90	0	5	0
5	90	5	6	0.1
10	95	10	7	1
15	115	25	19	3.9
20	130	45	30	7.3
30	161	85	57	9
40	178	90	50	7
50	180	115	53	6.3
60	178	120	60	5.9
75	161	120	50	5.5
90	145	119	55	4.2
120	125	100	45	3
150	115	85	32	2.3
180	100	80	20	2
210	95	75	10	1.9
240	90	70	9	1.5
260	90	65	8	1
280	90	60	7	0.8
300	90	60	6	0.7
360	90	50	5	0.5
420	90	40	5	0.2

Table 2: Given Parameter Values for Cobelli Data

RM*		FM	
Parameter	Value	Parameter	Value
S_G^*	0.0118 ± 0.004	p_2	0.011 ± 0.004
p_2^*	0.039 ± 0.004	p_2^*	0.043 ± 0.005
V^*	1.6 ± 0.04	S_I	0.001224 ± 0.000068
S_I^*	0.000924 ± 0.000063	p_3(calculated from S_I)	0.0000092855
p_3^*(calculated from S_I^*)	0.0000225225	S_I^*	0.000964 ± 0.00008
		p_3^*(calculated from S_I^*)	0.0000259075

values [11] (See Table 3). However, since the visual justification is strong, we accept this estimate of V^* and move on to FM. In general, we are not concerned with p_2, p_3, p_2^*, p_3^* from RM, RM* since these values are estimated again in FM.

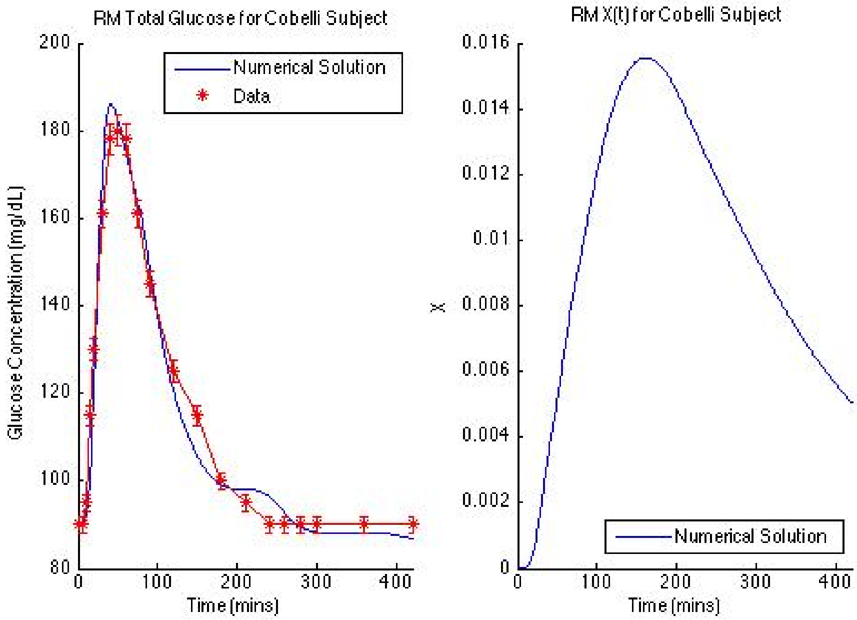

Figure 3.3: RM for Cobelli Test Subject

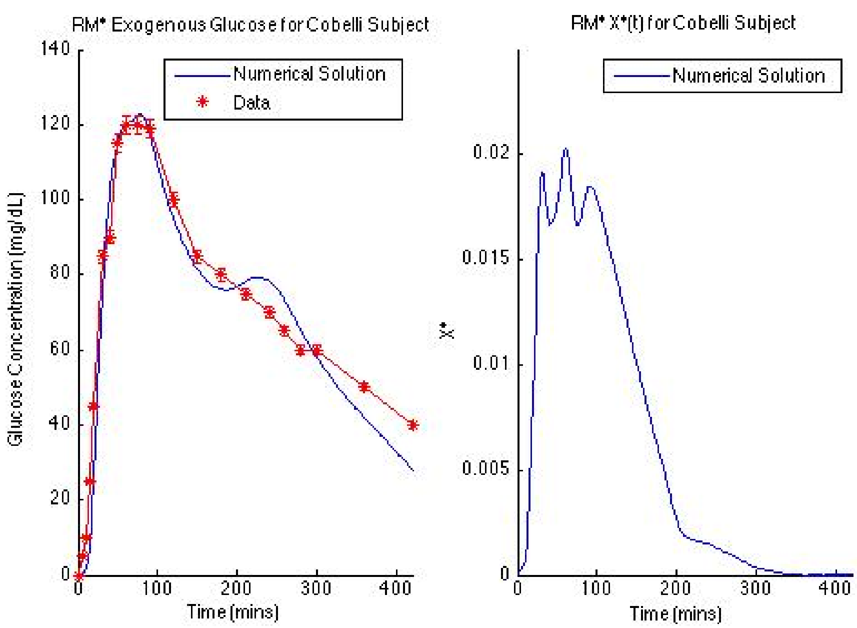

Figure 3.4: RM* for Cobelli Test Subject

The results from FM are presented in Figure 3.5 and Table 3 show good agreement with the estimated data values. We selected the partition of time for numerical Ra_{meal} by estimating the values used in [11]. The that numerical estimate for Ra_{meal} is slightly lower than the estimated Steele's Ra_{meal} from [11]. This may reflect error in the estimate of D (we estimated $D = 1000$; this value was not specified [11]).

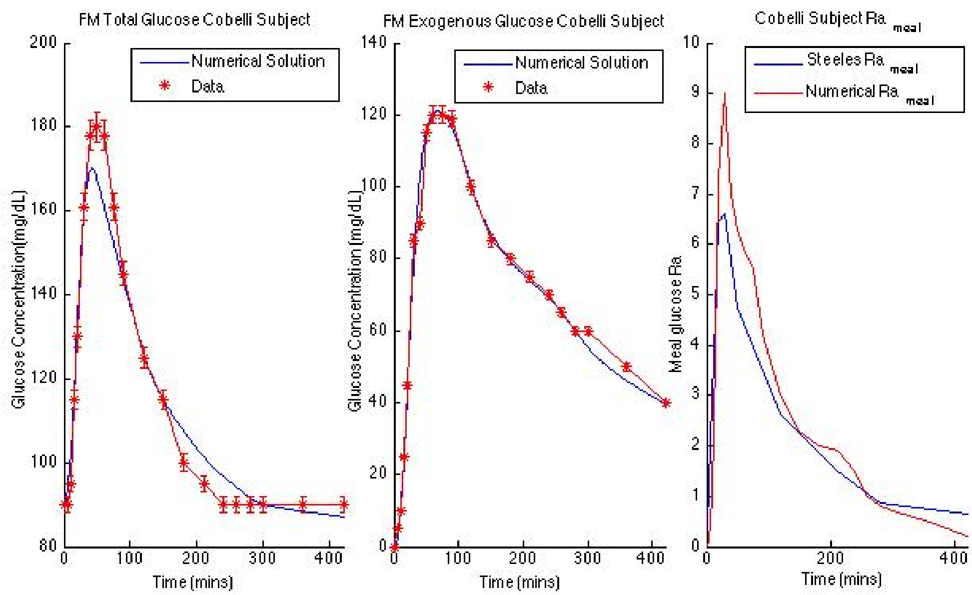

Figure 3.5: FM for Cobelli Test Subject

Table 3 presents the parameter results. The magnitude of parameters p_3 and p_3^* agrees with the reported value and the parameter p_2^* is exactly in the given range. Our estimated p_2 is smaller than the reported value. However, this discrepancy may result from not being able to check RM estimates against Cobelli RM values, a potential underestimate of V, and a potential overcontraint on α_i based on our estimate for D. Any combination of the previous limitations could lead to an inaccurate p_2 value. Since p_2 is small, our estimated $S_I = \dfrac{p_3}{p_2}V$ is high when compared to values from [11]. However, our estimated $S_I^* = \dfrac{p_3^*}{p_2^*}V$ is within the range described by [11]. The agreement of our parameter estimates with those in [11], together with the small error associated with FM, provide reasonable validation of our numerical implementation of OMM*.

Table 3: Estimates for Cobelli Data

RM		RM*	
Parameter	Value	Parameter	Value
S_G	0.0645	S_G^*	0.0139
p_2	0.0053	p_2^*	1.9516
p_3	0.0000	p_3^*	0.0007
V	1.0013	V^*	1.3976
Error	23.7776	Error	36.7399
Time (s)	42.57	Time (s)	148.43
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	5.278474568735358e-61	p_2	1.340043409308344e-06
10	2.903784643135438e+00	p_3	1.370298840337559e-06
20	6.437412953535223e+00	p_2^*	4.999687735453524e-02
30	6.594216958507896e+00	p_3^*	1.191701895480053e-05
50	4.738451343189025e+00		
60	4.436926140724022e+00		
120	2.628652797024872e+00		
210	1.487723579769383e+00		
280	8.622615009139135e-01	S_I	1.023907299792765e+00
420	6.370848193983463e-01	S_I^*	2.386651269203589e-04
Error (Total Glucose)	28.6097		
Error (Meal Glucose)	17.3874		
Time (s)	32.559034		

3.5.3 RESULTS

This section will explore the data from each subject individually in order to determine any outliers in our population. We then consider the average concentrations computed using four representative subjects.

Subject 1: Subject 1 is in the Obese Control Group. This subject's glucose concentrations are larger than any other subject in the experiment to date. Figure 3.6 shows Subject 1 RM/RM* solutions. Figures 3.7 and 3.8 show the FM solution and numerical Ra_{meal}, respectively. Parameter values for RM/RM* and FM can be found in Table 4.

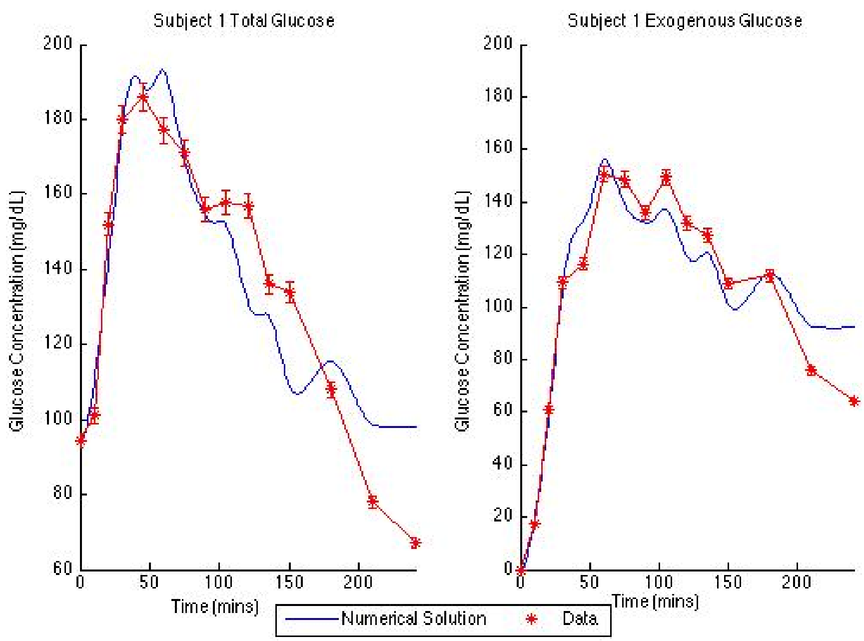

Figure 3.6: RM/RM* for Subject 1

It is immediately clear that V, V^* estimates for Subject 1 are significantly lower than the Cobelli test case. This trend was observed for all of our subjects. One possible explanation of this difference is that the magnitude of our Steele's Ra data is 3 times less than the Cobelli Ra data, which affects the value of V, V^* in RM, RM*. Once V, V^* are fixed at these lower values, we see relative agreement with numerical Ra_{meal} in FM. In general, our numerical Ra_{meal} wants to behave like exogenous glucose, as seen in the Cobelli test case, but our Steele's Ra_{meal} does not behave this way. To summarize, error in estimating V, V^* and subsequently S_G, S_G^* in RM, RM* is due to discrepancies between changes in Steele's Ra_{meal}, calculated from tracer data and exogenous glucose dynamics.

Table 4: Subject 1 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	1.673113146650401e-01	S_G^*	2.217328845895088e-01
p_2	2.226872676389498e-09	p_s^*	2.080971108661180e-09
p_3	2.179910458769852e-05	p_3^*	2.095237072512212e-05
V	5.487745244972300e-02	V^*	4.067744813643938e-02
Error	5.735431870141528e+01	Error	4.405826903920079e+01
Time (s)	40.011476	Time (s)	38.282590
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	6.315139238380014e-22	p_2	6.875177575412857e-04
20	7.757400940629864e-01	p_3	1.264593958970538e-05
45	1.320851209166621e+00	p_2^*	7.815196854621611e-07
75	1.620451340536869e+00	p_3^*	6.977247776800736e-06
105	1.696258127349721e+00		
135	1.685905090191237e+00		
180	1.488192561095683e+00	S_I	1.009394944209064e-03
240	7.903231230739882e-01	S_I^*	4.899346622022569e-01
Error (Total Glucose)	33.6329		
Error (Meal Glucose)	31.0981		
Time (s)	46.496048		

Subject 2: Subject 2 is part of the Obese Control Group. She is an obvious outlier due to noisy tracer-to-tracee enrichment data, which is used to calculate both exogenous glucose and Ra_{meal}.

The calculation of exogenous glucose and Ra_{meal} both use the noisy tracer-to-tracee enrichment data so both values are scaled in a noisy manner. RM* focuses on only exogenous glucose and Ra_{meal}, and since both of these values share the same noisy behavior, it is possible to produce a small error when fitting this data (error was 11.76). This idea does not translate when fitting total glucose since this value does not share the same pattern as Ra_{meal} (error was 64.39). Both of these ideas can be seen in Figure 3.9.

Even though subject 2 is an outlier in our population, we continue with the numerical imple-

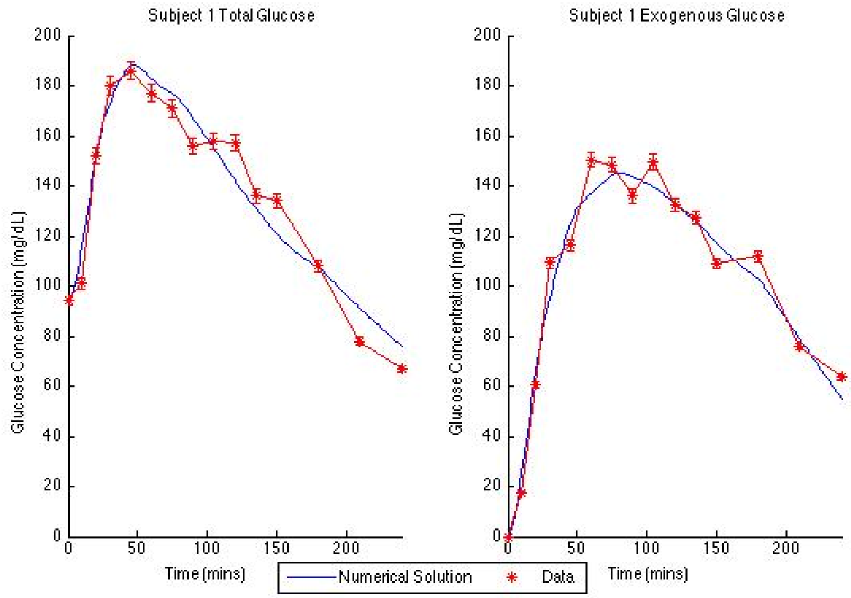

Figure 3.7: FM for Subject 1

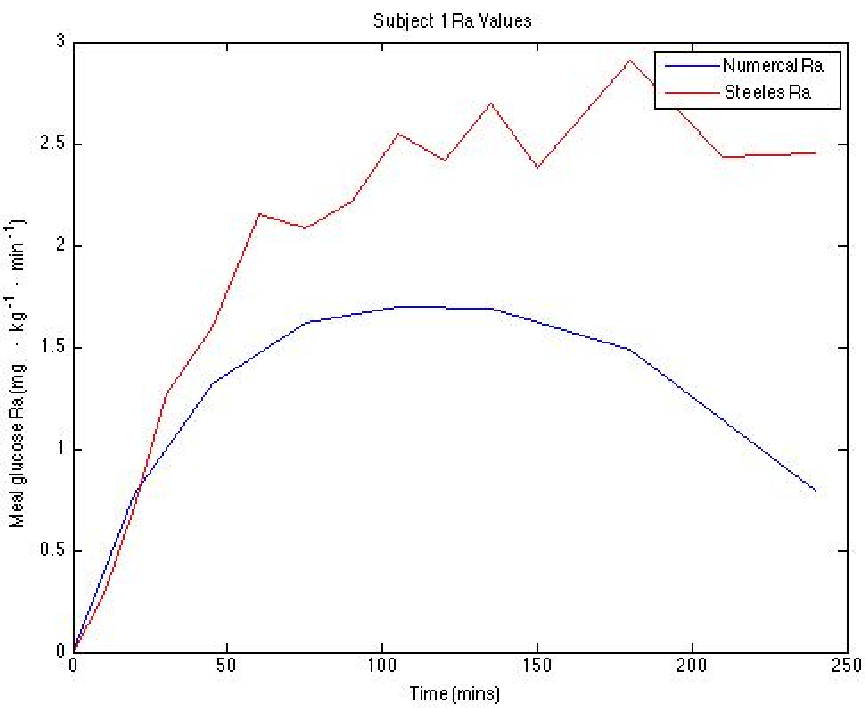

Figure 3.8: Numerical Ra_{meal} for Subject 1

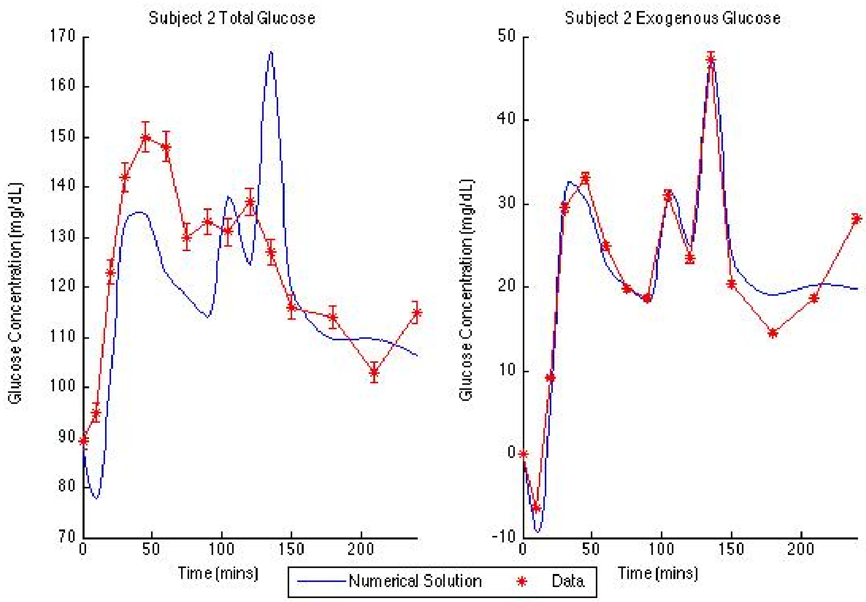

Figure 3.9: RM/RM* results for Subject 2

mentation for completeness. Figure 3.10 shows the results from FM, Figure 3.11 shows numerical Ra_{meal}, and Table 5 has parameter results for RM, RM*, FM. We can see that the numerical solution for total glucose has improved from FM (39% improvement), to the detriment of the exogenous glucose numerical solution (106% decrease).

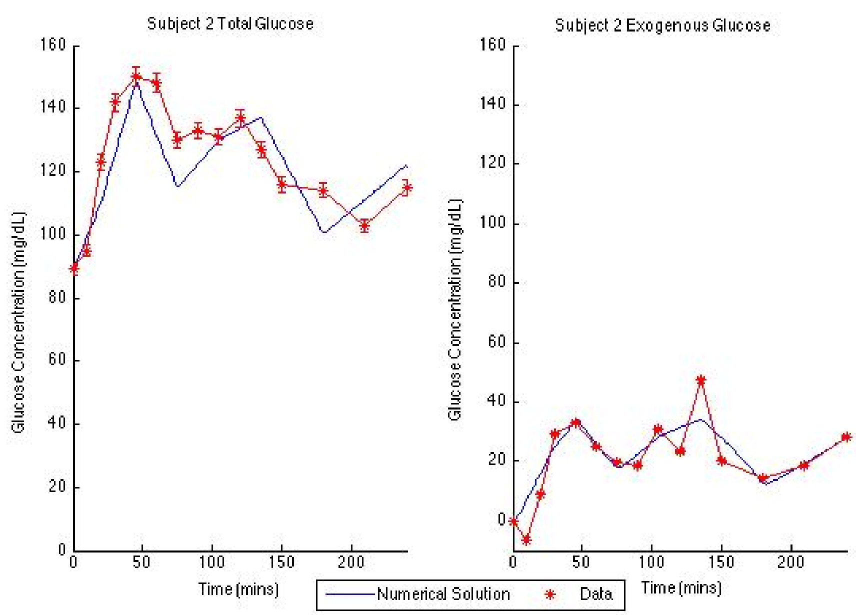

Figure 3.10: FM results for Subject 2

Table 5: Subject 2 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	2.836444781050041e+00	S_G^*	4.300666964511896e-01
p_2	5.088866372740366e-09	p_2^*	8.163751853816804e-03
p_3	2.112421680801738e-05	p_3^*	3.214966087612505e-05
V	5.539756229101681e-03	V^*	4.188885708943995e-02
Error	6.439421929257325e+01	Error	1.176310713391883e+01
Time (s)	1457.234109	Time (s)	259.054330
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	2.298183256249235e-13	p_2	6.719867700859917e-03
20	3.412824153801318e-01	p_3	1.563848534410686e-05
45	1.028069144947594e+00	p_2^*	3.042126862470667e-02
75	5.177953887535087e-01	p_3^*	4.966544587195229e-05
105	7.761217845612123e-01		
135	9.081427336964429e-01		
180	2.844556471346176e-01	S_I	1.289212830598513e-05
240	6.428149147568246e-01	S_I^*	9.044148241628860e-06
Error (Total Glucose)	39.1751		
Error (Meal Glucose)	24.2467		
Time (s)	204.929475		

When we consider numerical Ra_{meal} compared to Steele's Ra_{meal}, we can see that the numerical Ra_{meal} appears to be more biologically significant than Steele's Ra_{meal}. For example, the peak at $t = 135$ appears to be an outlier in Steele's Ra_{meal} while the numerical Ra_{meal} does not have any obvious outliers.

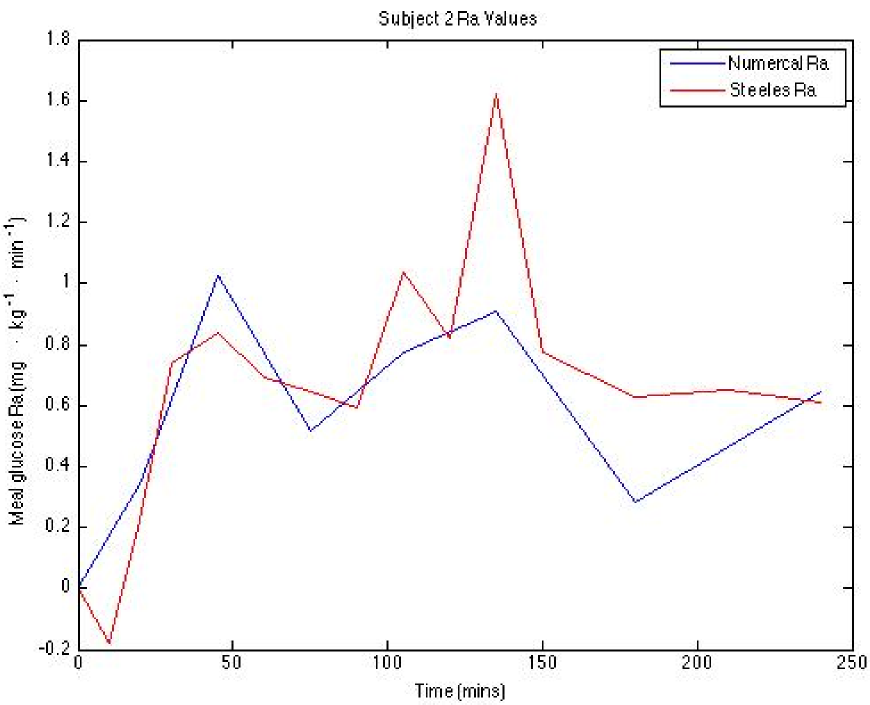

Figure 3.11: Numerical Ra_{meal} results for Subject 2

Subject 3: Subject 3 is the last subject in the Obese Control Group. Results from RM/RM* and FM provide good fits to the data. Parameter values from RM, RM*, FM can be found in Table 6 and agree with other subjects' parameter results. Figure 3.12 shows RM, RM* solutions, 3.13 shows FM solution, and Figure 3.14 shows numerical Ra_{meal}.

Subject 4: Subject 4 is the first subject in the Obese PCOS Group. RM/RM* and FM provide good fits, and S_I and S_I^* are in the range of reported values of [11]. The numerical solution for RM, RM* can be found in Figure 3.15; FM in Figure 3.16. Although the error in both total and exogenous glucose FM estimates increased compared to RM, RM* (12.07% and 70.84% increase, respectively), FM provides a reasonable fit to the data.

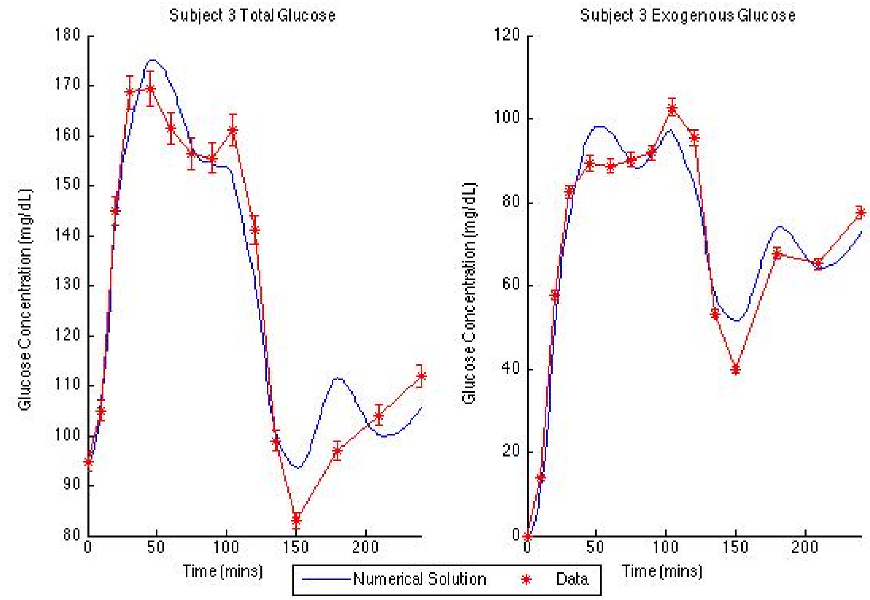

Figure 3.12: RM/RM* results for Subject 3

Table 6: Subject 3 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	5.313662565599957e-01	S_G^*	3.497897314653042e-01
p_2	1.429545109301413e-03	p_2^*	3.970854376961488e-03
p_3	2.130659737514779e-05	p_3^*	1.614695887454232e-05
V	3.109555082234151e-02	V^*	4.403883839263187e-02
Error	2.757278664295405e+01	Error	2.442521328016889e+01
Time (s)	98.849306	Time (s)	101.723046
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	5.945545691488047e-04	p_2	1.350979451120928e-02
20	1.059616081991209e+00	p_3	2.914109043545942e-05
45	1.900540479839384e+00	p_2^*	2.972749319357905e-02
75	1.622523395957593e+00	p_3^*	3.149886031159489e-05
105	3.009750158915595e+00		
135	1.410855062807524e+00		
180	1.351897259963057e+00	S_I	6.707417036598338e-05
240	1.331723415886752e+00	S_I^*	3.294843615931245e-05
Error (Total Glucose)	38.1691		
Error (Meal Glucose)	34.3303		
Time (s)	14.634140		

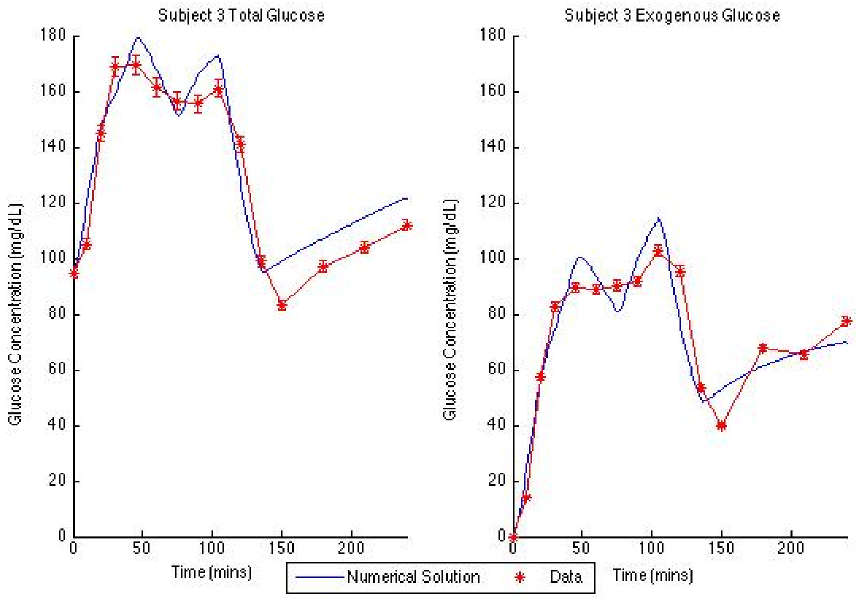

Figure 3.13: FM results for Subject 3

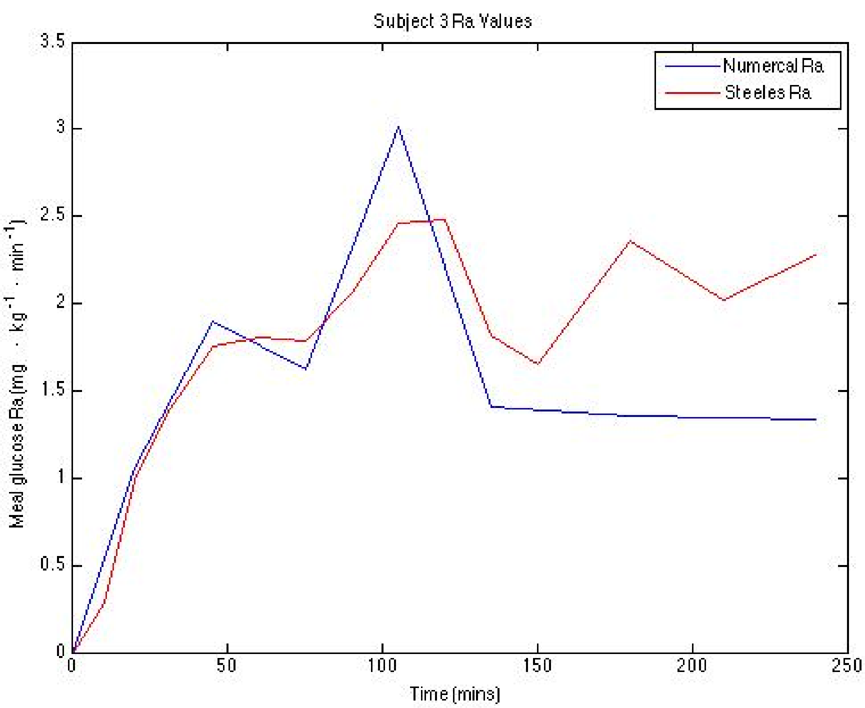

Figure 3.14: Numerical Ra_{meal} results for Subject 3

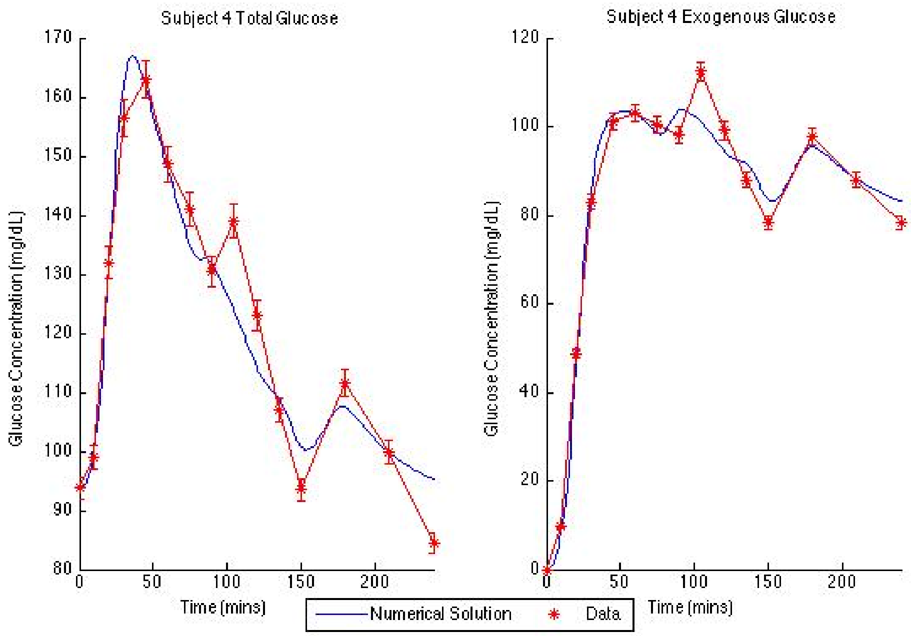

Figure 3.15: RM/RM* results for Subject 4

Table 7: Subject 4 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	2.292138736496028e-01	S_G^*	3.017026574124569e-01
p_2	2.036505426946322e-09	p_2^*	1.358472262341269e-09
p_3	1.779553379016973e-05	p_3^*	1.589354202203482e-05
V	4.758388399962413e-02	V^*	3.526749739879706e-02
Error	2.386344185285145e+01	Error	1.646284288405077e+01
Time (s)	22.687383	Time (s)	65.146664
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	3.985831174315762e-10	p_2	1.148725500196262e-03
20	4.912452937453880e-01	p_3	1.871808780479731e-05
45	1.617352115158830e+00	p_2^*	1.270761074507877e-03
75	1.787800885921826e+00	p_3^*	1.727466261330971e-05
105	2.092065286168347e+00		
135	1.852498144936253e+00		
180	2.192639844808783e+00	S_I	7.753630598833926e-04
240	1.867319604955663e+00	S_I^*	6.468529438098379e-04
Error (Total Glucose)	26.7383		
Error (Meal Glucose)	28.1156		
Time (s)	35.332711		

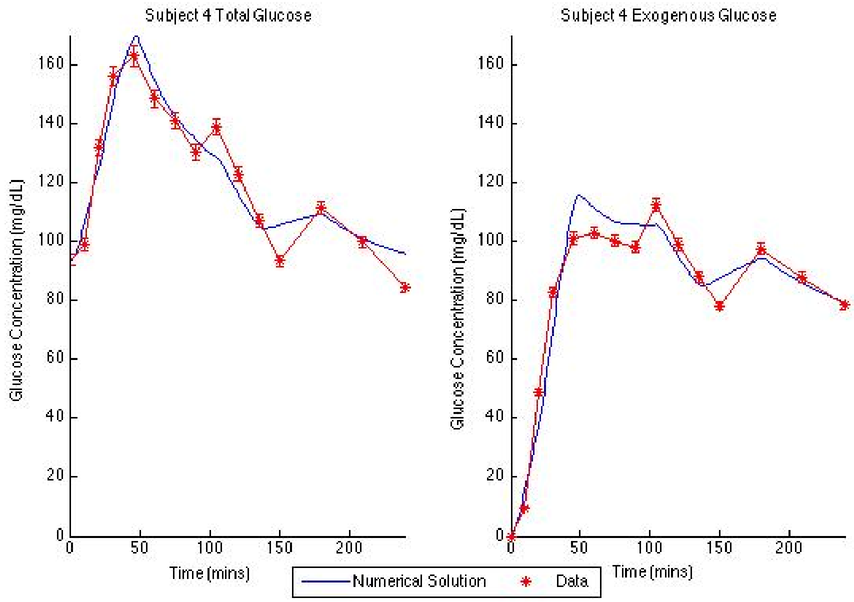

Figure 3.16: FM results for Subject 4

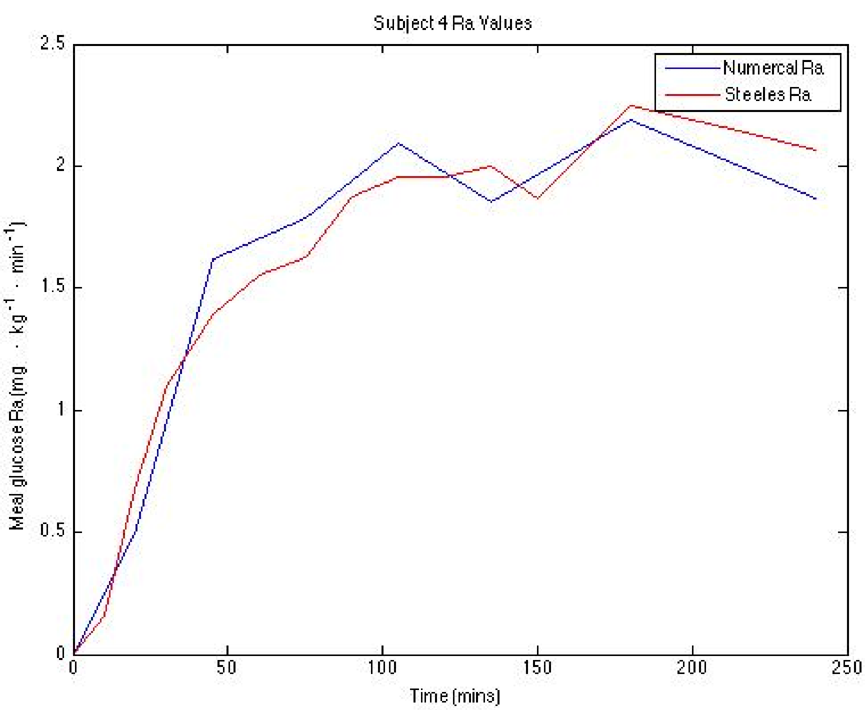

Figure 3.17: Numerical Ra_{meal} results for Subject 4

Subject 5: Subject 5 is in the Obese PCOS group. This subject's data has similar issues to those of Subject 2, so we classify this subject as an outlier. For completeness, we include RM, RM*, FM results for Subject 5.

As we can see from Figure 3.18, the RM solution for total glucose does not prove a good fit to the data. This is due to the noisy behavior of tracer-to-tracee enrichment data, which results in noisy exogenous glucose and Steele's Ra_{meal}.

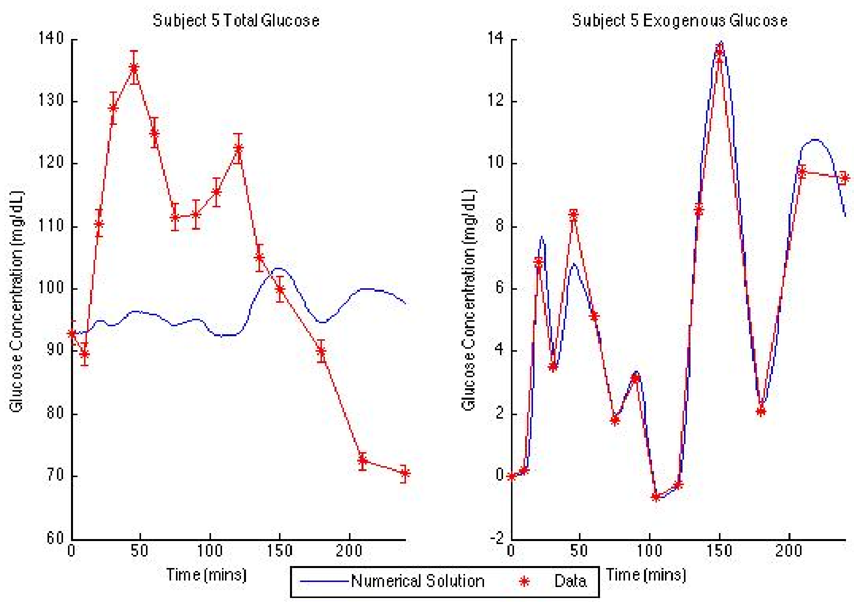

Figure 3.18: RM/RM* results for Subject 5

The numerical solutions for FM and Ra_{meal} are shown in Figure 3.19 and 3.20, respectively. Again, the solution for total glucose does not provide a good fit to the data.

Subject 6: Subject 6 is in the Obese PCOS Group. This subject has some of the more interesting data since she exhibits hyperinsulemia, where her total glucose concentrations dip below basal vaues at $t = 90$. The numerical solution for RM and RM* are given in Figure 3.21. Figure 3.22 shows the numerical solution for FM and Figure 3.23 shows numerical Ra_{meal}. FM improved our solution overall from RM, RM* (6.82% improvement in total glucose, -2.71% in exogenous glucose).

Table 8: Subject 5 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	4.638222300089152e+00	S_G^*	2.875557890304166e-01
p_2	2.328316123377006e-01	p_2^*	5.776412094491674e-03
p_3	7.140713648375771e-11	p_3^*	8.892727950473256e-05
V	1.123414887033968e-02	V^*	3.387127103041024e-02
Error	8.596326194157341e+01	Error	2.288969298691674e+00
Time (s)	184.455379	Time (s)	250.166900
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	4.078898893860925e-23	p_2	4.833326303188328e-02
20	2.197256674757407e-04	p_3	5.661487140326470e-05
45	1.501950911605829e-01	p_2^*	6.031634589774948e-03
75	2.825992120996602e-02	p_3^*	3.870696566807326e-05
105	2.440875342119849e-04		
135	2.113333153887250e-01		
180	1.193024762413172e-01	S_I	1.315905142178914e-05
240	1.796747038299203e-01	S_I^*	7.209319599224705e-05
Error (Total Glucose)	91.6309		
Error (Meal Glucose)	10.9992		
Time (s)	800.690086		

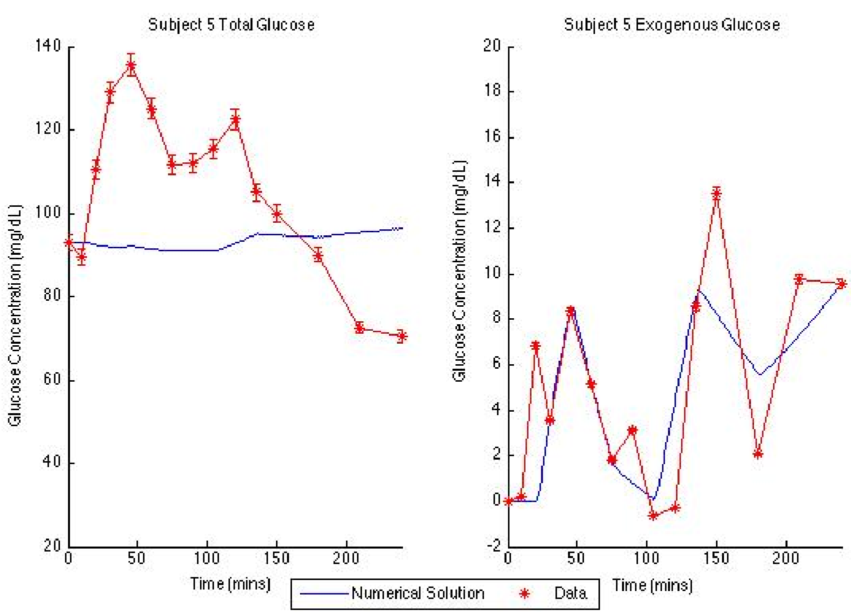

Figure 3.19: FM results for Subject 5

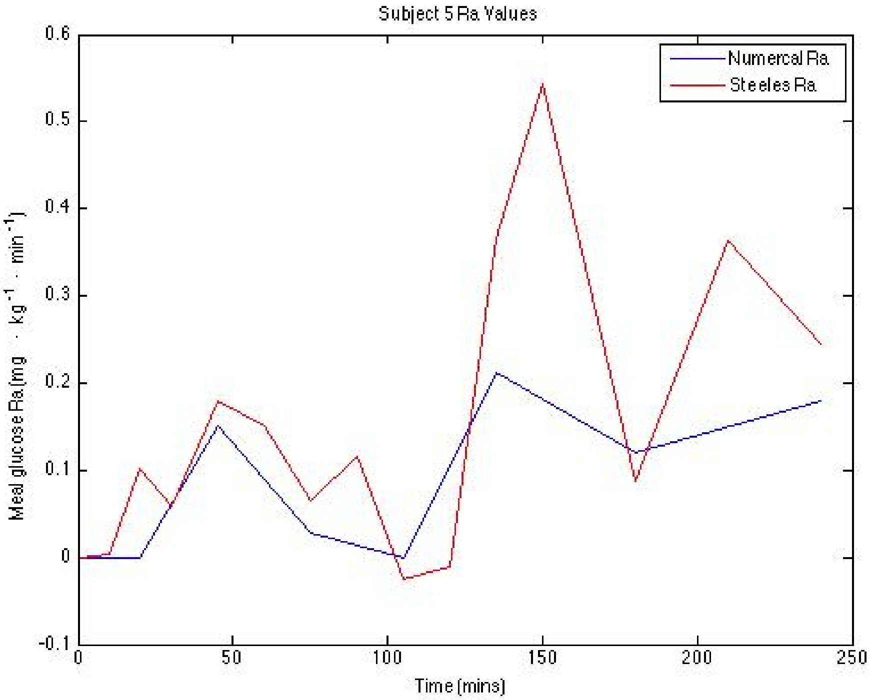

Figure 3.20: Numerical Ra_{meal} results for Subject 5

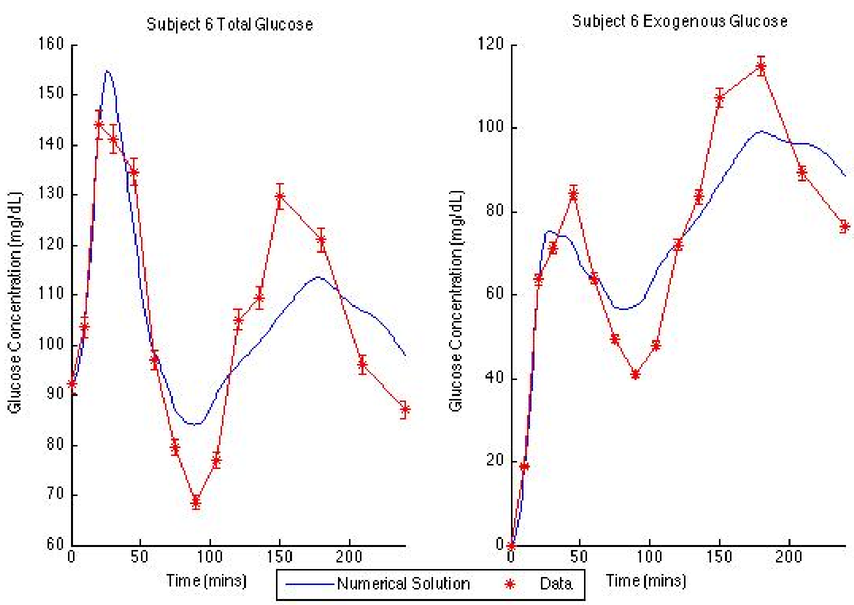

Figure 3.21: RM/RM* results for Subject 6

Table 9: Subject 6 RM/FM Parameter Results

RM		RM*	
Parameter	Value	Parameter	Value
S_G	6.621899406459641e-02	S_G^*	2.098488626403128e-01
p_2	2.184672177878457e-03	p_2^*	5.936659359936876e-03
p_3	9.549446211760252e-06	p_3^*	1.949848633650202e-05
V	7.670112141761820e-02	V^*	3.982850579277993e-02
Error	4.188070055900822e+01	Error	4.150709894727523e+01
Time (s)	114.425259	Time (s)	31.569186
FM			
Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	4.867734806071132e-50	p_2	7.164318877135931e-07
20	7.228090412308747e-01	p_3	2.868344715674283e-06
45	3.982906638471131e-01	p_2^*	4.242694339025013e-06
75	3.213403537748639e-01	p_3^*	1.026756563450777e-10
105	5.384507129236348e-01		
135	7.598275433945474e-01		
180	9.618666149028328e-01	S_I	3.070846790567032e-01
240	5.353090443165711e-01	S_I^*	1.856211490778105e-06
Error (Total Glucose)	39.025		
Error (Meal Glucose)	42.6336		
Time (s)	51.959339		

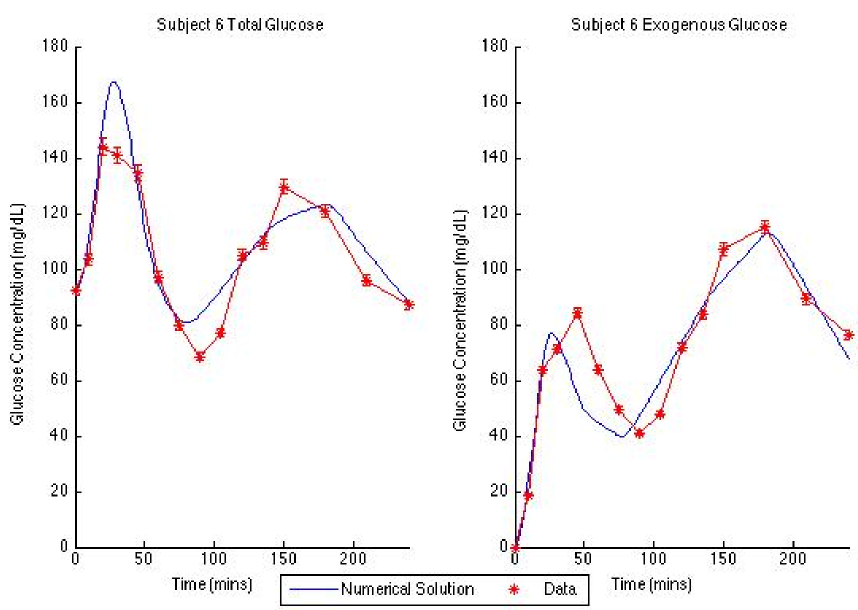

Figure 3.22: FM results for Subject 6

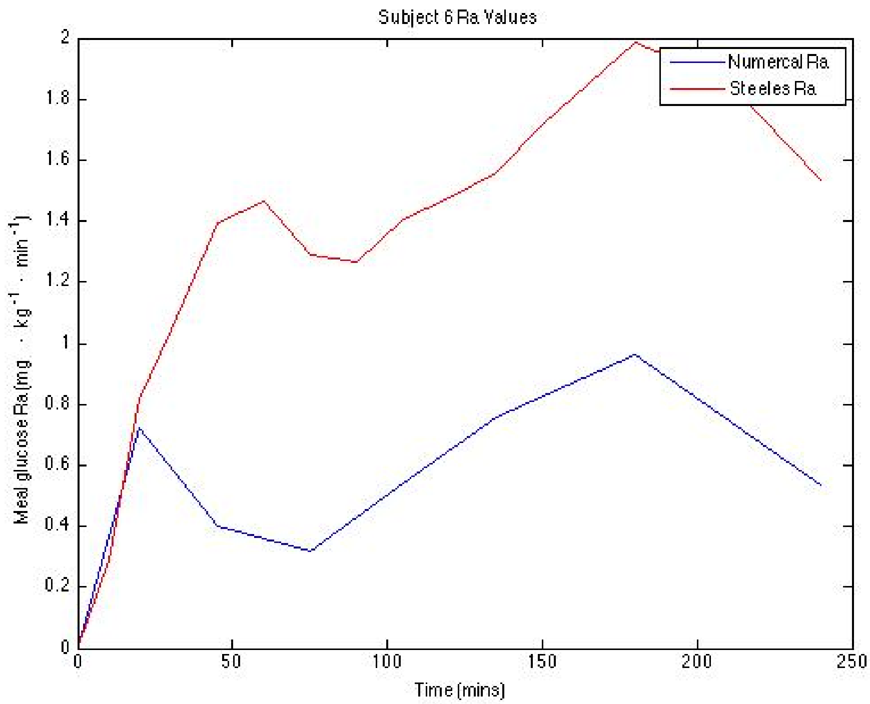

Figure 3.23: Numerical Ra_{meal} results for Subject 6

Average Subject (Subjects 1,3,4,6): Lastly, we will consider averaged data from subjects 1,3,4,6. The results from RM and FM can be found in Figure 3.24 and Table 10. S_I and S_I^* are in the range of reported values [11].

Table 10: Parameter Values from RM/FM for Average Subject

RM			
S_G	2.07E-01	S_G^*	4.71E-01
p_2	3.69E-09	p_2^*	2.75E-10
p_3	1.50E-05	p_3^*	2.07E-05
V	5.08E-02	V^*	2.49E-02
FM			
p_2	1.31E-03	p_2^*	2.65E-3
p_3	1.55E-05	p_3^*	2.16E-05
S_I	6.04E-04	S_I^*	4.14E-04

By averaging the non-outlier subjects from our study, we minimize subject specific outliers and smooth out the data. FM and numerical Ra_{meal} provide excellent fits to the group averaged data.

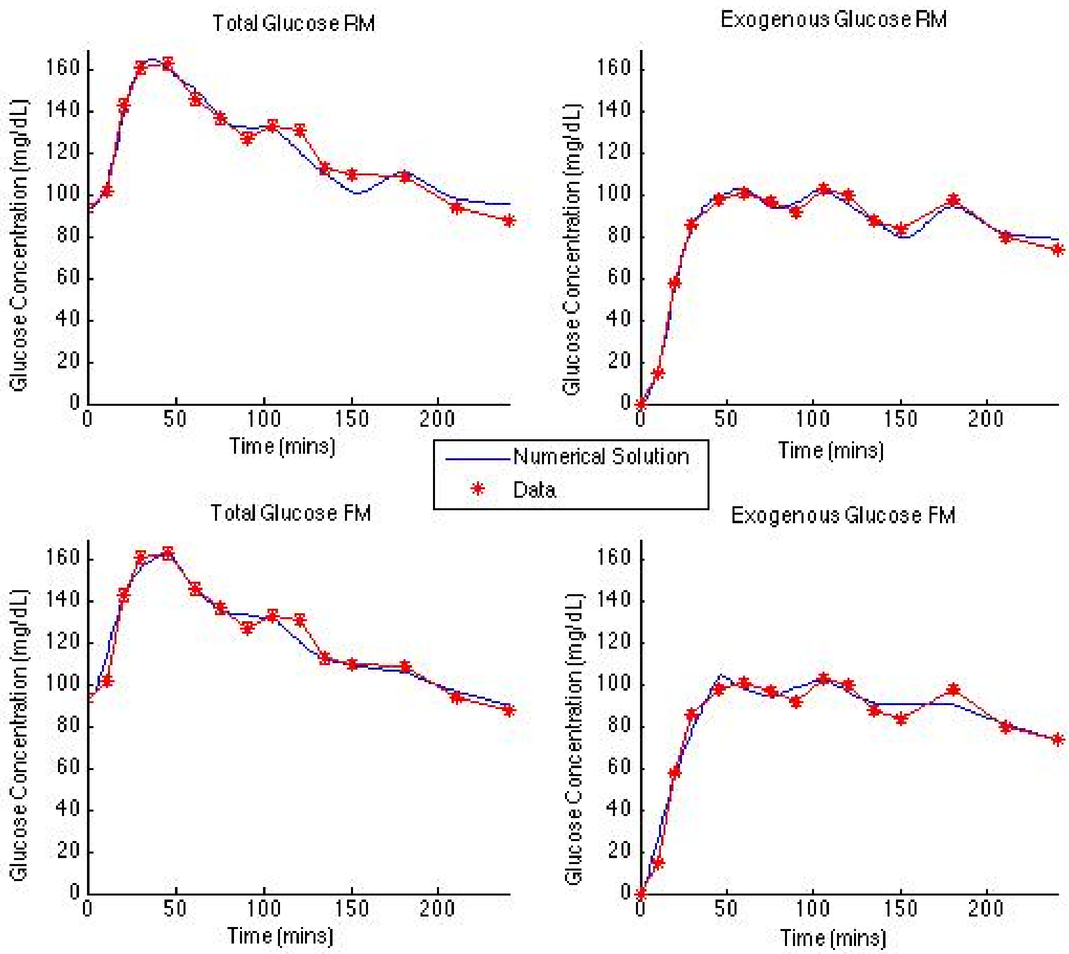

Figure 3.24: RM/FM for Average Subject

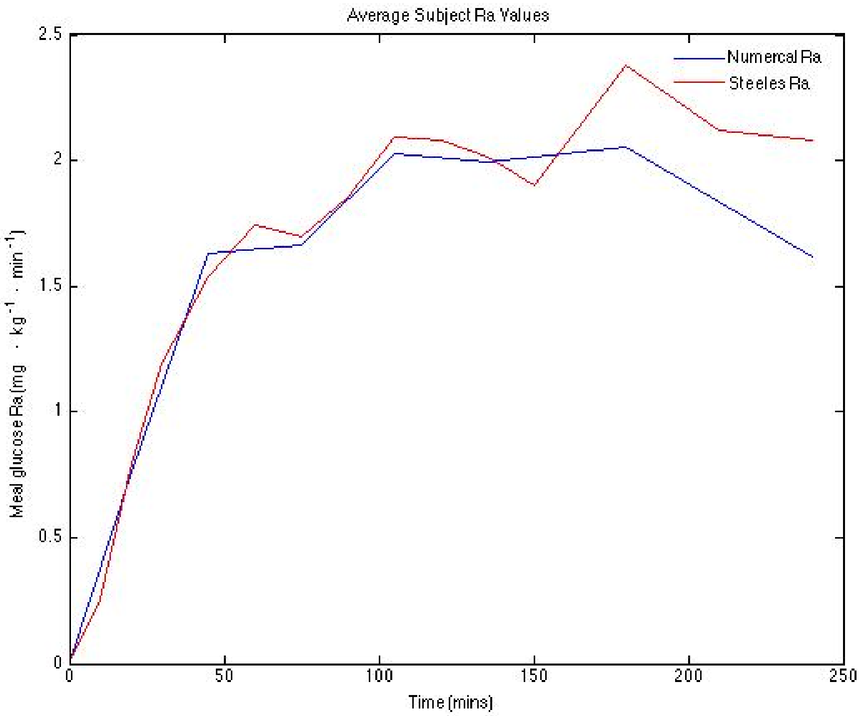

Figure 3.25: RM/FM: for Average Subject

Averaging V, V^*, S_G, S_G^*: It is often suggested in the literature [11, 12, 23] that mean values for V, V^*, S_G, S_G^* should be used to overcome the identifiability issues. So far in this section, we have considered each subject independently, and averaged group data. We will now explore the idea of using mean values for V, V^*, S_G, S_G^* from RM, RM*. These values are shown in Figure 3.26 and Figure 3.27.

As we can see from Table 11, there is quite a spread for each parameter value. The spread gives us a sense of how well the mean represents the data; if the spread is large, the mean is not a good representation of the data. By computing the Coefficient of Variation (CV), we can determine how much variability there is within our data for V, V^*, S_G, S_G^*. From this value, we can see that the mean value for S_G is not reliable since the addition of just one subject would change this value drastically. We claim that since CV for V is close to 1 (0.72), that V also has large variability. The only parameters that the mean may be representing well are V^* and S_G^*. V^* is the only parameter for which we can reliably use the mean value. The mean value for S_G^* is also a reasonable estimate for representing the data, but it is not as accurate as the average value for V^*. Unfortunately, because there is such a spread in our small population, model results using averaged V, V^*, S_G, S_G^*

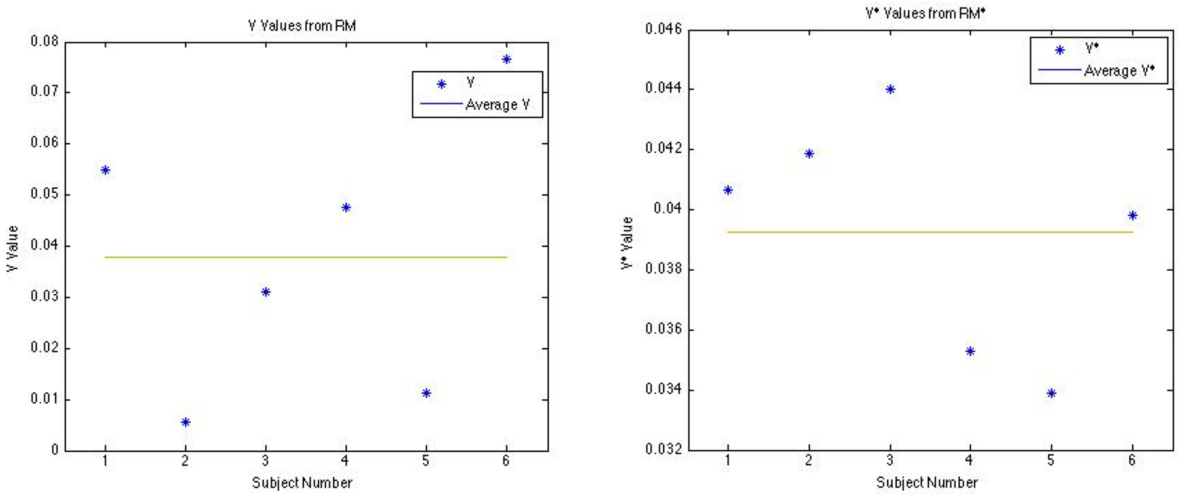

Figure 3.26: Average V and V^* Values

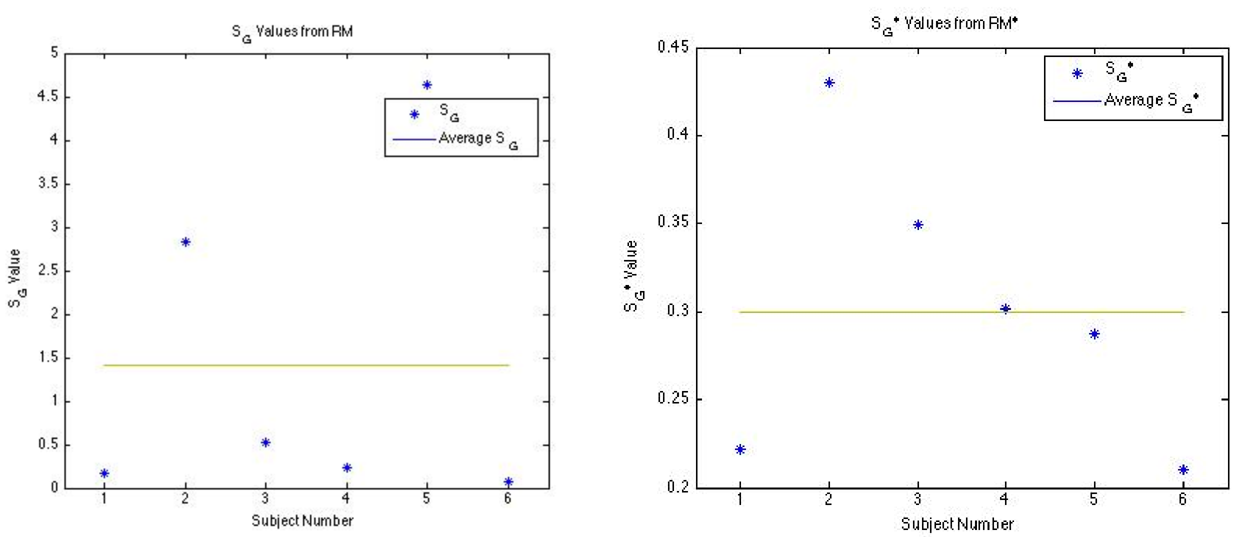

Figure 3.27: Average S_G and S_G^* Values

did not provide good fits for the data from individual subjects (results not shown).

Table 11: Mean V, V^*, S_G, S_G^*

	V	V^*	S_G	S_G^*
Mean	0.0378	0.0393	1.4115	0.3001
Standard Deviation	0.0272	0.0039	1.8959	0.0822
Variance	7.3841e-04	1.5412e-05	3.5945	0.0068
Interquartile Range	0.0436	0.0066	2.6691	0.1281
Range	0.0712	0.0102	4.5720	0.2202
Coefficient of Variation (Standard Deviation/Mean)	0.7196	0.0992	1.3432	0.2739

Additionally, we considered V, V^*, S_G, and S_G^* averaged over Subjects 1, 3, 4, 6, we omitted values from Subjects 2 and 5 since their RM values did not produce good results. Mean parameter values can be found in Table 12. As we can see, S_G still has quite a bit of spread in its values across the 4 subjects. Here, we will compute the numerical solution for FM for these 4 subjects.

Table 12: Mean V, V^*, S_G, S_G^* without Subjects 2 and 5

	V	V^*	S_G	S_G^*
Mean	0.0526	0.0400	0.2485	0.2708
Standard Deviation	0.0189	0.0036	0.2002	0.0666
Variance	3.5788e-04	1.3063e-05	0.0401	0.0044
Interquartile Range	0.0264	0.0048	0.2635	0.1100
Range	0.0456	0.0088	0.4651	0.1399
Coefficient of Variation (Standard Deviation/Mean)	0.3593	0.0900	0.8056	0.2459

Table 13 shows the parameter values from FM for Subject 1 using the average V, V^*, S_G, S_G^* from Subjects 1, 3, 4, 6. For this subject, the error for the numerical result using average values increases compared to the error using subject-specifc values from RM, RM* (-13.82% total glucose error, -8.07% exogenous glucose error). However, this decrease in accuracy of the numerical solution is small, and the solution still provides a good fit to the data. Figure 3.28 shows the numerical solution from FM. Note that the S_I value for this subject is outside the range of typical S_I values [11]. S_I^* is within the range of typical reported values [11].

Subject 3 FM parameter values are found in Table 14. For this subject, using the average values for V, V^*, S_G, S_G^* has improved the numerical solution from FM for exogenous glucose compared to

Table 13: Subject 1 FM Parameter Results using Average V, V^*, S_G, S_G^*

FM Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	1.541400262735745e-85	p_2	1.027429579327787e-07
20	9.880636316779648e-01	p_3	1.376584289514701e-05
45	1.512494099665063e+00	p_2^*	1.688502245508061e-03
75	2.031393155206310e+00	p_3^*	9.527394292307040e-06
105	2.039806632503653e+00		
135	2.028805579537440e+00		
180	1.688425717135725e+00	S_I	7.047522777750631e+00
240	1.031222180172400e+00	S_I^*	2.967961346267323e-04
Error (Total Glucose)	38.282 (old:33.6329)		
Error (Meal Glucose)	33.6072 (old:31.0981)		
Time (s)	89.805739		

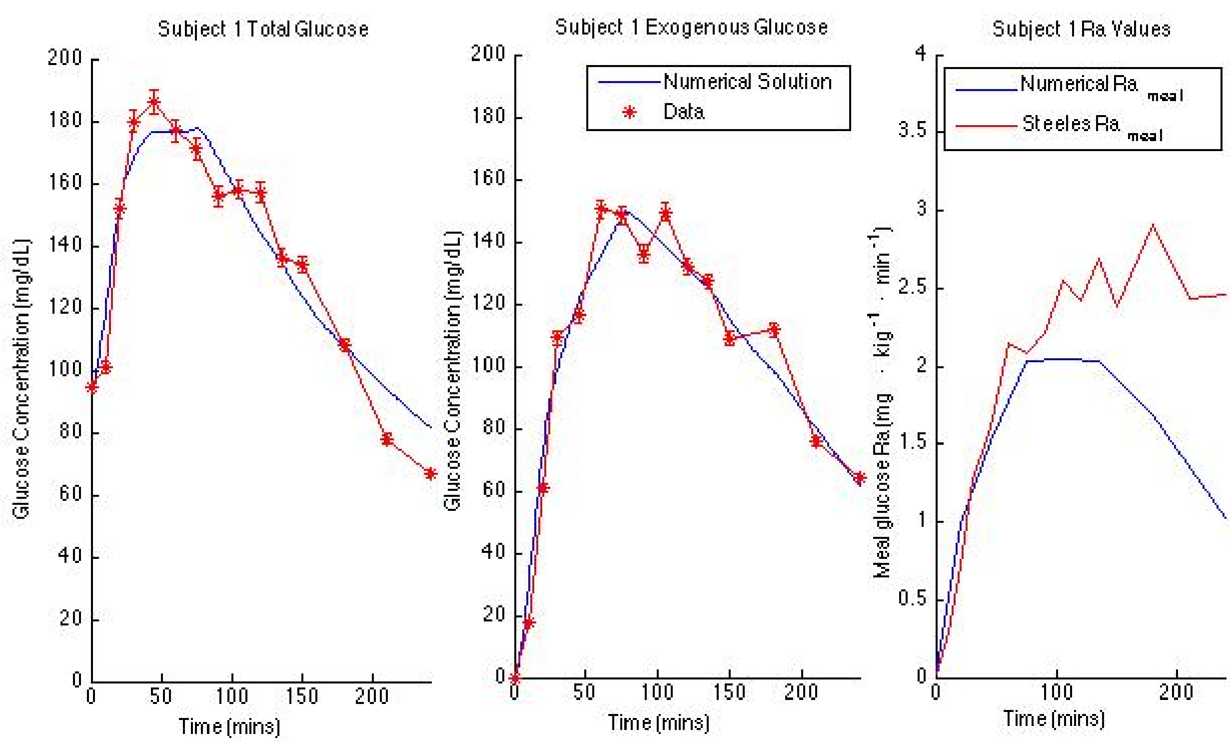

Figure 3.28: FM for Subject 1 using Average V, V^*, S_G, S_G^*

subject-specific FM numerical solution (-7.78% error total glucose, 8.05% error exogenous glucose). The numerical solution for FM is in Figure 3.29. The computed S_I and S_I^* values agree with reported values [11].

Table 14: Subject 3 FM Parameter Results using Average V, V^*, S_G, S_G^*

FM Parameter	Value	Parameter	Value
Ra Time	*Ra* Value		
0	4.571697373548058e-04	p_2	1.369347180751623e-02
20	8.009781586689256e-01	p_3	1.402877605898520e-05
45	1.268735575980358e+00	p_2^*	2.656374319569393e-02
75	1.114117362187322e+00	p_3^*	1.699085359323805e-05
105	1.957428585754432e+00		
135	9.318928079401788e-01		
180	8.887263312669134e-01	S_I	5.388798626638915e-05
240	1.031154335700614e+00	S_I^*	3.364431331911069e-05
Error (Total Glucose)	41.1383 (old:38.1691)		
Error (Meal Glucose)	31.5652 (old:34.3303)		
Time (s)	17.696125		

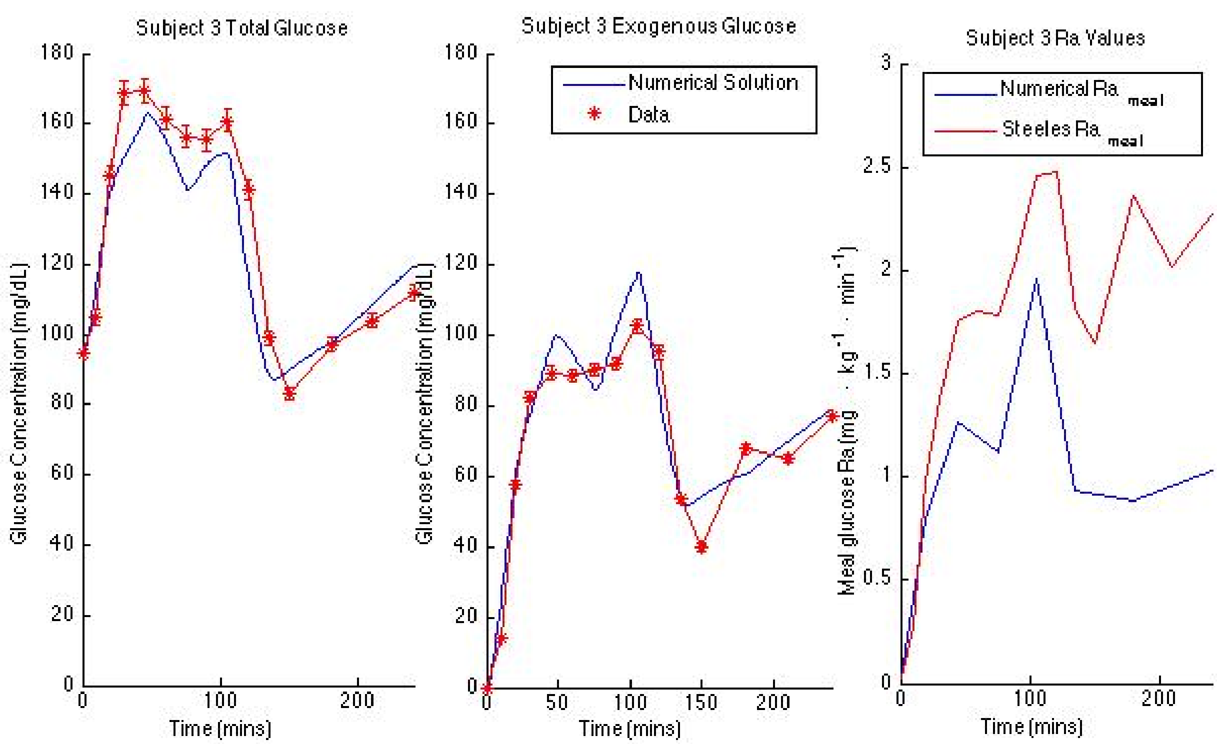

Figure 3.29: FM for Subject 3 using Average V, V^*, S_G, S_G^*

Subject 4 has the best results using the average values for V, V^*, S_G, S_G^* in terms of error. There was an overall increase in accuracy of the numerical result from FM (11.51% total glucose, 33.20% exogenous glucose). However, S_I and S_I^* have now moved outside the range of previously reported values [11]. When we used subject-specific RM, RM* parameter values for FM, the computed values for S_I and S_I^* were within the acceptable range. Here, those values are too large. In this situation, average V and V^* are larger than subject-specific V and V^* for Subject 4. Also, p_2 and p_2^* are too large. This leads to S_I and S_I^* being outside the range of reported values for this case. The numerical solution for FM is in Figure 3.30.

Table 15: Subject 4 FM Parameter Results using Average V, V^*, S_G, S_{G^*}

FM Parameter	Value	Parameter	Value
Ra Time	*Ra* Value		
0	2.753026751522251e-06	p_2	1.625486296087834e-05
20	7.048667894136079e-01	p_3	1.444375235200540e-05
45	1.512048697826308e+00	p_2^*	2.166812361256966e-06
75	1.553686488749751e+00	p_3^*	1.317918312126242e-05
105	2.195405214346399e+00		
135	1.688208088838938e+00		
180	2.205015966760451e+00	S_I	4.673932813484826e-02
240	1.899435236068206e+00	S_I^*	3.199285016891190e-01
Error (Total Glucose)	23.6598 (old:26.7383)		
Error (Meal Glucose)	18.7807 (old:28.1156)		
Time (s)	52.052384		

The final subject we consider with mean values for V, V^*, S_G, S_G^* is subject 6. The parameter values from FM are in Table 16. The accuracy of the numerical result for total glucose decreased (-49.26%), but the accuracy of the numerical solution for exogenous glucose increased (16.82%). Additionally, the computed S_I and S_I^* values are within the appropriate range. The numerical solution from FM is in Figure 3.31.

Finally, we eventually want to use computed values (S_I, S_I^*) to determine if there is a difference between the Obese Control Group and Obese PCOS Group. See Table 17 for a review of S_I and S_I^* values. As we can see, there is no real distinction between groups (Subject 1 and 3 are Obese Control, Subject 4 and 6 are Obese PCOS). We conclude that averaging V, V^*, S_G, S_G^* is possible and would allow for an accurate way to compare subjects. However, using such a small population leads us to inconclusive results. It is impossible to know what the “true” mean values from RM, RM* should be without a large subject population.

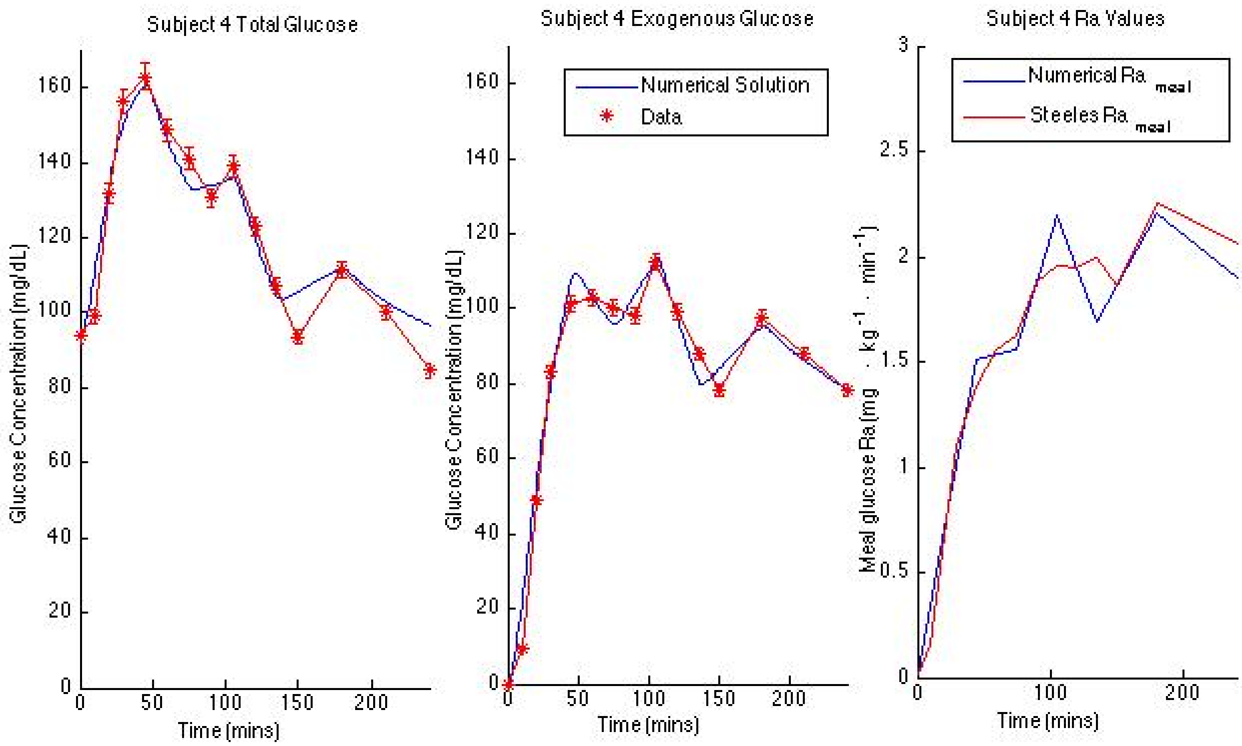

Figure 3.30: FM for Subject 4 using Average V, V^*, S_G, S_G^*

Table 16: Subject 6 FM Parameter Results using Average V, V^*, S_G, S_G*

FM Parameter	Value	Parameter	Value
Ra Time	*Ra* Value		
0	1.824362788917089e-03	p_2	9.817646593479395e-03
20	8.690740352830372e-01	p_3	1.163956317946263e-05
45	1.405840753292841e+00	p_2^*	2.454463737788987e-02
75	8.060599269406968e-01	p_3^*	1.606745658496330e-05
105	4.787949586486163e-01		
135	1.010844108451711e+00		
180	1.469192757022670e+00	S_I	6.236128153628668e-05
240	8.843428766904885e-01	S_I^*	3.443311071812331e-05
Error (Total Glucose)	58.2477 (old:39.025)		
Error (Meal Glucose)	35.4644 (old:42.6336)		
Time (s)	13.581835		

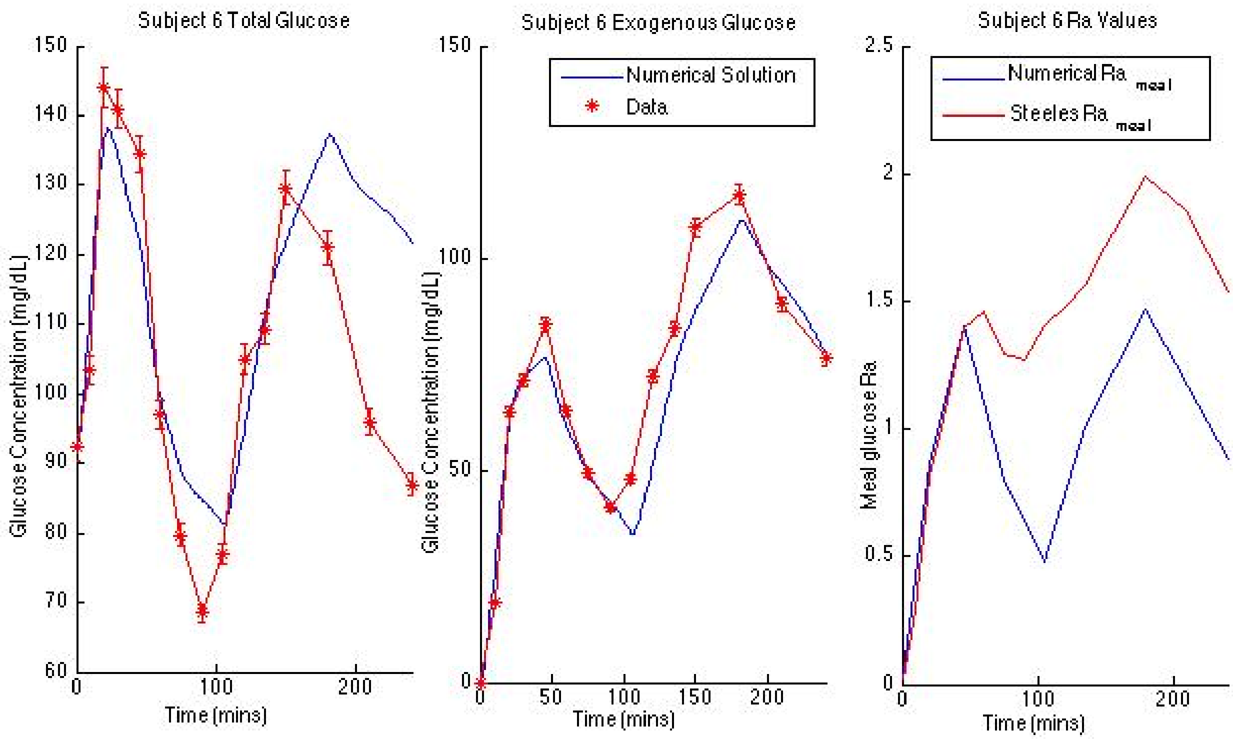

Figure 3.31: FM for Subject 6 using Average V, V^*, S_G, S_G^*

Table 17: Subjects 1, 3, 4, 6 S_I, S_I^* Values using Average V, V^*, S_G, S_G*

Subject #	S_I	S_I^*
1	7.047522777750631e+00	2.967961346267323e-04
3	5.388798626638915e-05	3.364431331911069e-05
4	4.673932813484826e-02	3.199285016891190e-01
6	6.236128153628668e-05	3.443311071812331e-05
Mean	1.773594588788320e+00	8.007334381194574e-02

3.6 OTHER METRICS

In this section, we will explore other metrics such as area under the curve for the numerical solution, time to maximum glucose, and comparing insulin concentrations. We will use the numerical solution from averaging values of V, V^*, S_G, S_G^* estimated for Subjects 1, 3, 4, 6. The numerical results from FM with other metrics included for Subjects 1 and 3 (Obese Control) are in Figure 3.32 and Subjects 4 and 6 (Obese PCOS) are in Figure 3.33. Insulin data for all 4 subjects are shown in Figure 3.34. These results are summarized in Table 26.

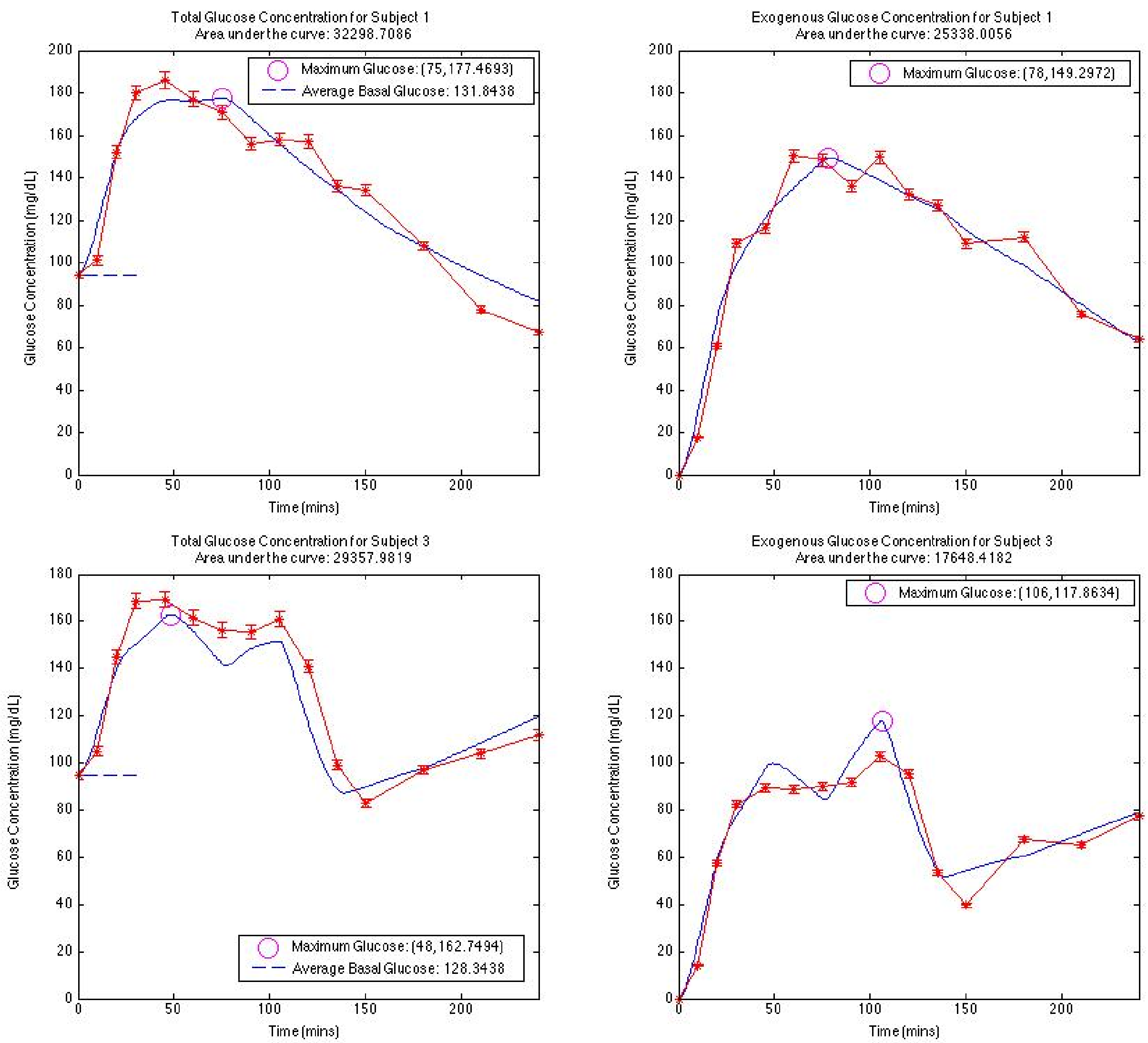

Figure 3.32: Other Metrics for Obese Control Group

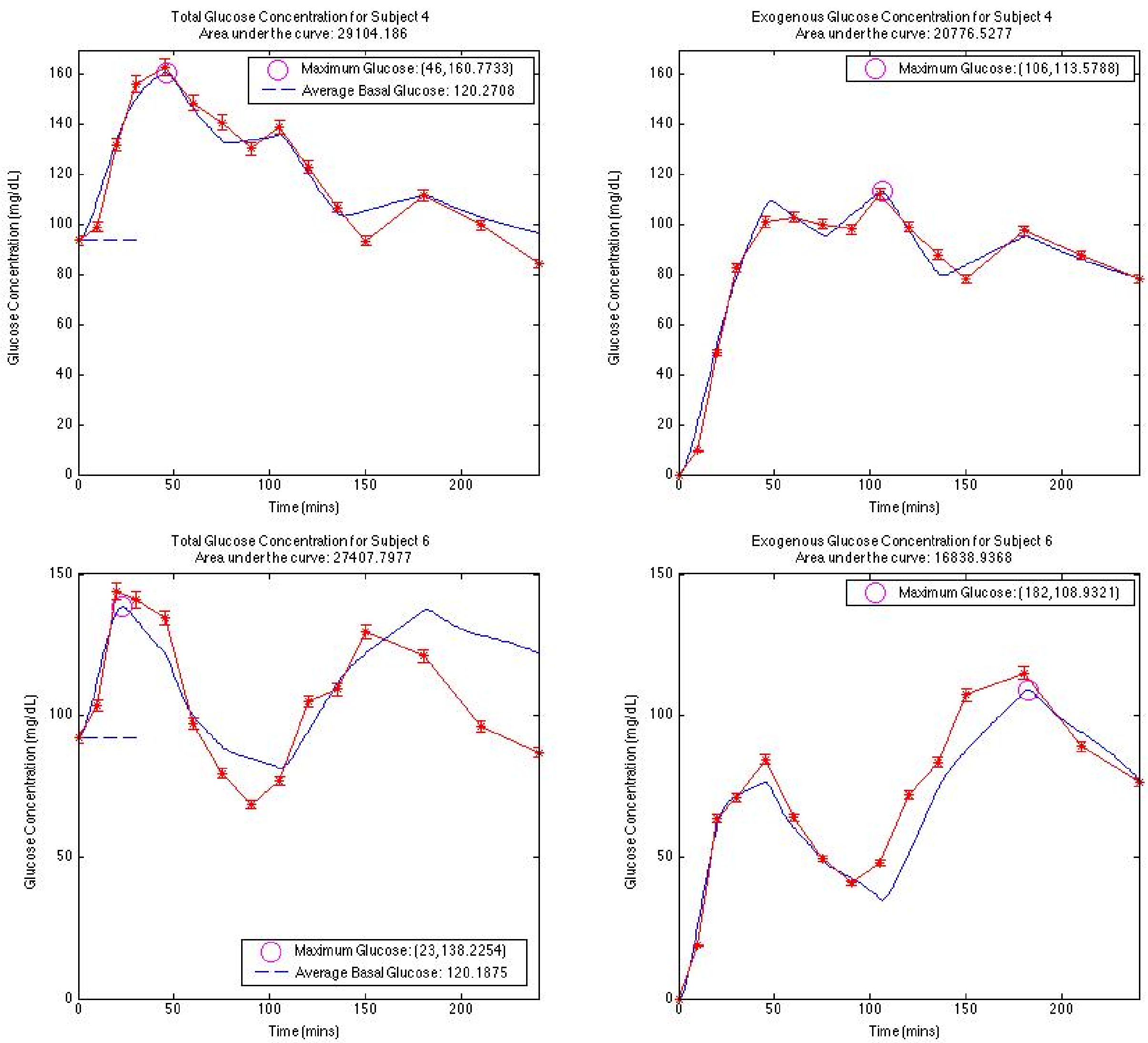

Figure 3.33: Other Metrics for Obese PCOS Group

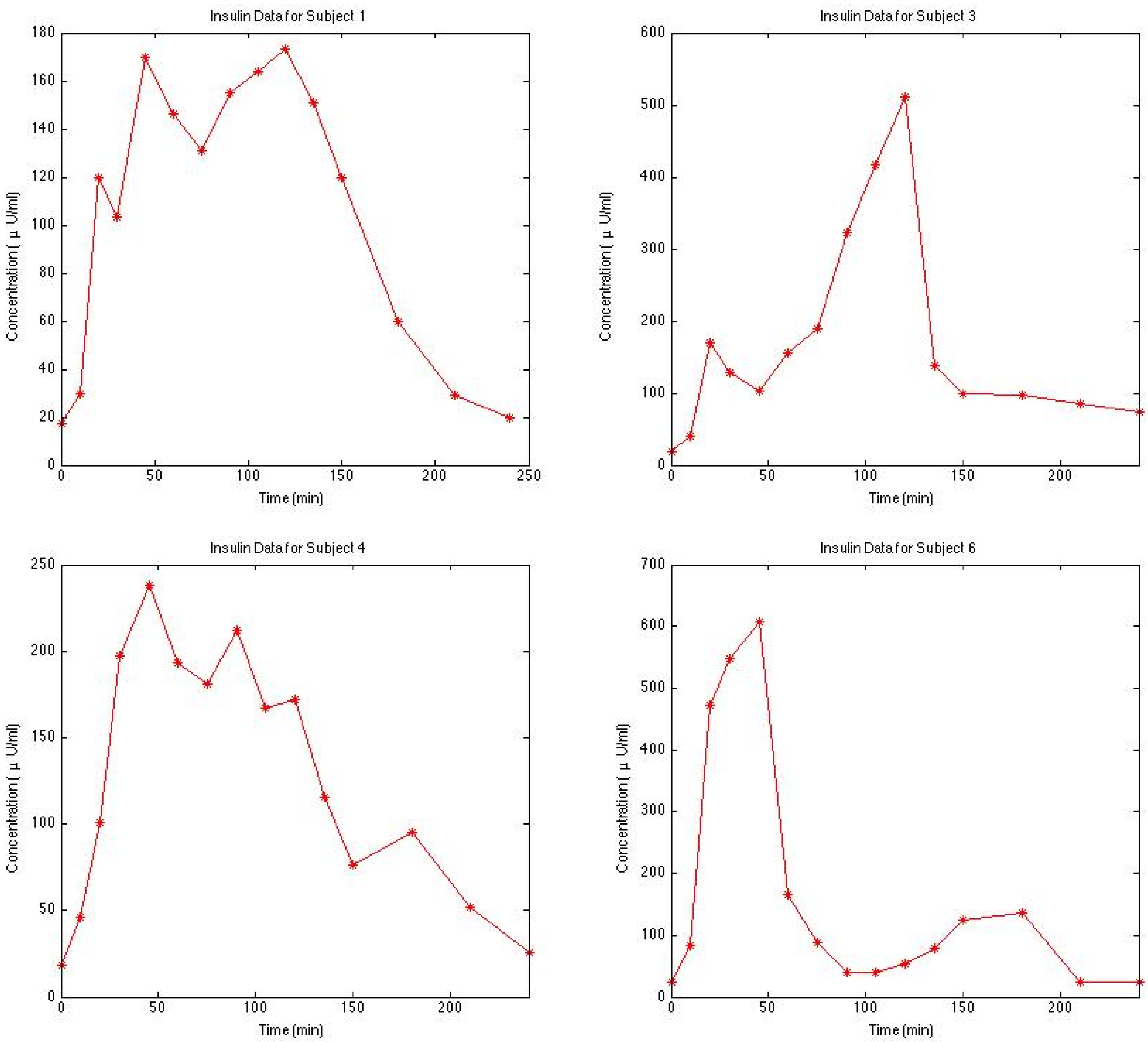

Figure 3.34: Insulin Data for Subjects 1, 3, 4, 6

Table 18: Subjects 1, 3, 4, 6 Glucose Other Metics

Subject #	Average Basal Glucose (mg/dL)	Time to Peak Total Glucose (mins)	Peak Total Glucose (mg/dL)	Time to Peak Exogenous Glucose (min)	Peak Exogenous Glucose (mg/dL)	Time to Peak Insulin (min)	Peak Insulin Concentration (μ U/ml)	Area Under Numerical Solution (Total) (mins · mg/dL)	Area Under Numerical Solution (Exogenous) (mins · mg/dL)
1	120.2708	46	160.7733	78	149.2972	120	173	32298.7086	25338.0056
3	128.3438	48	162.7494	106	117.8634	120	510	29357.9819	17648.4182
4	120.2708	46	160.7733	106	113.5788	45	238	29104.1860	20776.5277
6	120.1875	23	138.2254	182	108.9321	45	608	27407.7977	16838.9368
Mean	122.2682	40.75	155.6303	118	122.4179	82.50	382.25	29542.16855	20150.472075
· Control	124.3073	47	161.7613	92	133.5803	120	341.50	30828.34525	21493.2119
· PCOS	120.2292	34.50	149.4993	144	111.2554	45	423	28255.99185	18807.73225
$t-$test ($\alpha = 0.05\%$)									
h	0	0	0	0	0	1	0	0	0
p	0.41872	0.39205	0.39183	0.32779	0.29517	0	0.77557	0.26891	0.59756

Given the very small sample size available to date, we do not expect to be able to rigorously compare the Obese Control and Obese PCOS groups. However, to show the intended comparison, we used **ttest2**, a built-in MATLAB function that applies student's $t-$test. For this test, at $p = 0.05$, the probability that the difference between the two groups occurring by chance is only 5%. The function **ttest2** returns $h = 1$ if the test rejects the null hypothesis at the 5% significance level; 0 otherwise. The results of this test can be found in Table 26. Interestingly, the metric "Time to Peak Insulin" is significantly different between the two groups, suggesting that this metric may be of interest when diagnosing an individual with PCOS. We note that this may be coincidental; with such a small subject population and a limited description of the time values associated with insulin concentrations (lack of numerical solution means we can only use measured data), the results may not be robust. Additional subjects are needed to confirm this result and identify other metrics that may be useful for diagnosing and characterizing PCOS.

3.7 GLUCOSE CONCLUSION

In this chapter, we discussed the most commonly used model to describe subject-specific glucose/insulin dynamics during an Oral Glucose Tolerance Test (OGTT). Specifically, we derived OMM* (§3.2) and explained how model parameters are used to quantify hepatic insulin resistance. We then investigated the structural identifiability of this model using the Taylor Expansion Method (§3.4) in order to determine if the parameters are uniquely identifiable. The identifiability analysis found that the model is system unidentifiable. Using this result, we developed a structurally and numerically identifiable approach using a Reference Model (RM, RM*) and a Final Model (FM), and we implemented this numerical approach for each subject to determine subject-specific parameter values (§3.5). We found low S_I^* values in our cohort compared to published data suggesting that hepatic insulin resistance may be implicated in obese adolescent females. A low S_I^* implies more insulin is needed to achieve the same action on glucose disposal. In this case, hepatic tissue is not responding appropriately to high insulin concentrations, so hepatic IR is indicated (see Table 17 for S_I^*)[4]. Given our small sample size, we were unable to conclude if there are differences in hepatic IR between Obese Control and Obese PCOS groups. Lastly, we considered other metrics in order to identify measures that may be able to categorize a subject as PCOS (§3.6). The re-

[4]The results from §3.2 - §3.5 were presented at The 1st Annual Meeting of SIAM Central States Section April 11, 2015 in Rolla, MO. The Journal of Computational and Applied Mathematics published a special issue of the proceedings of this meeting. A paper consisting of highlights from §3.2 - §3.5 has been submitted.

sults from this section found that the time to peak insulin may be a metric of interest; it was the only metric that showed a statistical significance between Obese Control and Obese PCOS using Subjects 1, 3, 4, 6 at the 5% level. As with S_I^*, additional subjects are needed to confirm that time to peak insulin ($t_{\text{max. insulin}}$) is a defining trait for PCOS. It is possible that these two metrics (S_I^* and $t_{\text{max. insulin}}$) together would give a more detailed view on PCOS and help to identify targeted therapeutics. For example, lower S_I^* values in obese adolescent females, coupled with insulin peaking at an earlier time in PCOS, may lead physicians to focus on treatments that are most effective directly after a meal in order to utilize the naturally occurring peak insulin concentration and help clear exogenous glucose in an effective manner. However, these are preliminarily findings and additional work is necessary to refine the clinical interpretation and translate these results to improved patient care.

CHAPTER 4
GLYCEROL (ADIPOSE IR)

As described previously (Introduction), glucose is the main energy source for the body. However, if glucose is not readily available, the body produces glucose from endogenous sources. The primary method for glucose production is the breakdown of glycogen stores in the liver, but a secondary source is gluconeogenesis, which converts noncarbohydrate carbon sources into glucose. The main substrates for this conversion are free fatty acids (FFA) and glycerol, both of which are released from adipose tissue through a process called lipolysis. Here, we focus on glycerol dynamics. This chapter will introduce a glycerol model describing glycerol dynamics during an OGTT, investigate the identifiability of the model parameters as we want them to be used as comparison metrics between our two groups: Obese Control and Obese PCOS. Specifically, we will compare S_g between the two groups. We will end the chapter with exploring other metrics related to glycerol to determine if adipose IR occurs in girls with PCOS.

4.1 PROPOSED GLYCEROL MODEL

While fasting, lipolysis releases glycerol from adipose tissue. After a meal, insulin should suppress lipolysis and mediate adipose uptake of excess glycerol. This glycerol clearance occurs in a concentration dependent manner. Define $g(t)$ as glycerol concentration at time t (Umol/L). Let $Ra(t)$ be the rate of appearance of glycerol into plasma (Umol/kg/min), and let $Rd(t)$ be the rate of disappearance of glycerol (Umol/kg/min). Then the change in glycerol concentration during an OGTT is given by

$$\frac{dg(t)}{dt} = \frac{Ra(t) - Rd(t)}{V} \tag{4.1}$$

where V is the distribution volume (L/kg). Since we assume the rate of disappearance is constant, we let $Rd(t) = Rd$. Define $S_g = \frac{Rd}{V}$. Then

$$\frac{dg(t)}{dt} = -S_g g(t) + \frac{Ra(t)}{V} \tag{4.2}$$

is the proposed model of glycerol dynamics during an OGTT. To our knowledge, glycerol dynamics during an OGTT have not been modeled previously.

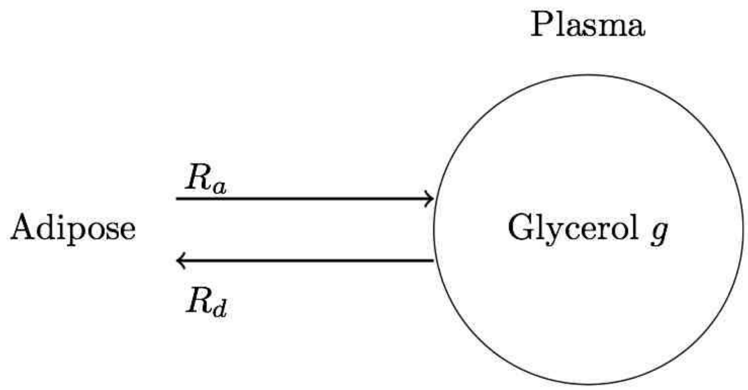

Figure 4.1: Proposed Glycerol Model

4.2 IDENTIFIABILITY ANALYSIS

We will use the Taylor Expansion method to determine the identifiability of the model parameters as described in §3.3. Consider the Glycerol Model, given by

$$\begin{cases} \dot{g} = -S_g g(t) + \dfrac{Ra(t)}{V} \\ y(t) = g(t) \end{cases} \tag{4.3}$$

where

$$Ra(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \leq t \leq t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}, \tag{4.4}$$

$y(t)$ is observed glycerol concentrations at t, and $\vec{p} = [S_g, V, \alpha_0, \ldots, \alpha_n]^T$ is the parameter vector. Since the Taylor Expansion method is a local analysis, we will consider breakpoints related to Ra individually.

Assume $t_i, i = 0, \ldots, n$ are fixed, with $t_0 = 0$. Existing glycerol physiology suggests that $g(t) > 0$ and $Ra(t) > 0$ for all t [26]. We assume $Ra(0) = R_b$ (drop the 'a' for convenience, 'b' refers to basal Ra value in the fasted state). We compute R_b by averaging Ra values calculated at times before $t = 0$, e.g., $t = -20, -10$. Technically, R_b is an average value, but for this analysis we assume this average to be the exact value. The biological assumption validating this claim is before the glucola drink is consumed, the body is in steady-state. Therefore, any deviations from a constant value for R_b is measurement error.

To begin, we will consider the identifiability of the model about $t_0 = 0$. Technically, we will consider t as $t \to t_0^+$ since Ra is not differentiable at $t_i, i = 0, \ldots, n$. Therefore, the interval of interest is $t_0 = 0 \le t \le t_1$.

Now we may start the method. First $y(t) = g(t)$ so $y(t_0^+) = g(t_0^+) := \lim_{t \to t_0^+} g(t) = g(0) = g_b$. Hence, g_b is a known constant. We note that this value should be known from the experimental data from $t < 0$; however, it is necessary to begin the method here to confirm that this value is unique on the interval $[0, t_n]$ in order for the remainder of the analysis to hold.

Next, we see that

$$\dot{y}(t) = \dot{g}(t) = -S_g g(t) + \frac{Ra(t)}{V},$$

hence,

$$\begin{aligned} \dot{y}(t_0^+) = \dot{g}(t_0^+) &= \lim_{t \to t_0^+} \left[-S_g g(t) + \frac{Ra(t)}{V} \right] \\ &= -S_g g_b + \frac{R_b}{V}. \end{aligned}$$

As $Ra(t)$ is continuous, we can evaluate $Ra(0)$ naturally: for $0 \le t \le t_1$, $Ra(t) = \alpha_0 + \frac{\alpha_1 - \alpha_0}{t_1 - t_0}(t - t_0) = \alpha_0 + \frac{\alpha_1 - \alpha_0}{t_1} t$, since $t_0 = 0$. So, $Ra(0) = \alpha_0 + \frac{\alpha_1 - \alpha_0}{t_1}(0) = \alpha_0$. It follows that $Ra(0) = R_b = \alpha_0 > 0$; the parameter α_0 is uniquely identifiable. Finally, rearranging for S_g, we have

$$S_g = \frac{1}{g_b} \left[\frac{\alpha_0}{V} - \dot{y}(t_0^+) \right], \tag{4.5}$$

or, $S_g = S_g(V)$. At this stage, we claim the parameters S_g and V are unidentifiable.

To confirm the relationship between S_g and V in (4.5), we must consider additional derivatives.

We have

$$\ddot{y}(t) = -S_g\dot{g}(t) + \frac{\dot{Ra}(t)}{V} = S_g^2 g(t) - \frac{S_g}{V}Ra(t) + \frac{\dot{Ra}(t)}{V}.$$

From the definition of $Ra(t)$, we see that $\dot{Ra}(t_0^+) = \lim_{t\to t_0^+} \dot{Ra}(t) = \frac{\alpha_1 - \alpha_0}{t_1 - t_0} = \frac{\alpha_1 - R_b}{t_1}$. Thus,

$$\ddot{y}(t_0^+) = S_g^2 g_b - \frac{S_g}{V}R_b + \frac{1}{V}\frac{\alpha_1 - R_b}{t_1}.$$

The third derivative is

$$\dddot{y}(t) = S_g^2\dot{g}(t) - \frac{S_g}{V}\dot{Ra}(t)$$

as $\ddot{Ra}(t) = 0$ for all t. Simplifying, we obtain

$$\dddot{y}(t) = -S_g^3 g(t) + \frac{S_g^2}{V}Ra(t) - \frac{S_g}{V}\dot{Ra}(t),$$

and

$$\dddot{y}(t_0^+) = -S_g^3 g_b + \frac{S_g^2}{V}R_b - \frac{S_g}{V}\frac{\alpha_1 - R_b}{t_1}.$$

If we continue taking derivatives, we arrive at the following relationship

$$y^{(k)}(t) = (-S_g)^k g(t) + \frac{(-S_g)^{k-1}}{V}Ra(t) + \frac{(-S_g)^{k-2}}{V}\dot{Ra}(t) \tag{4.6}$$

for $k \geq 2$. Taking the limit as $t \to t_0^+$, we have

$$y^{(k)}(t_0^+) = \lim_{t\to t_0^+} y^{(k)}(t) = y^{(k)}(0) = (-S_g)^k g_b + \frac{(-S_g)^{k-1}}{V}R_b + \frac{(-S_g)^{k-2}}{V}\frac{\alpha_1 - R_b}{t_1}. \tag{4.7}$$

We claim this gives no new information on the identifiability of the model since the only parameters are S_g, V, α_1 and they have the same dependencies as before.

We conclude that this model is system unidentifiable since there exists an infinite number of solutions for the parameter S_g given V. Therefore, if V is known, then by (4.5) and (4.7), S_g and α_1 are uniquely identifiable. Hence, the system becomes locally identifiable around $t = 0$ if V is known.

To establish the identifiability of the parameters $\alpha_2, \ldots, \alpha_n$, we apply this method around $t_i, i = 1, \ldots, n$. First, note that the limit $t \to t_1^+$ corresponds to the interval $t_1 \leq t \leq t_2$. Using the same method as before, we have $y(t) = g(t)$ so $y(t_1^+) = g(t_1^+) = \lim_{t \to t_1^+} g(t) = g_1$. Thus, g_1 is a unique constant value. Next,

$$\dot{y}(t) = \dot{g}(t) = -S_g g(t) + \frac{Ra(t)}{V},$$

and

$$\begin{aligned} Ra(t_1^+) &= \lim_{t \to t_1^+} \left[\alpha_1 + \frac{\alpha_2 - \alpha_1}{t_2 - t_1}(t - t_1) \right] \\ &= \alpha_1 \end{aligned}$$

so

$$\begin{aligned} \dot{y}(t_1^+) &= \lim_{t \to t_1^+} \left[-S_g g(t) + \frac{Ra(t)}{V} \right] \\ &= -S_g g_1 + \frac{\alpha_1}{V}. \end{aligned}$$

In this limit, the parameter of interest, α_2, drops out, so we take another derivative to obtain

$$\ddot{y}(t) = S_g^2 g(t) - \frac{S_g}{V} Ra(t) + \frac{\dot{Ra}(t)}{V}.$$

Taking the limit as $t \to t_1^+$, we have

$$\begin{aligned} \ddot{y}(t_1^+) &= S_g^2 g_1 - \frac{S_g}{V} Ra(t_1^+) + \frac{\dot{Ra}(t_1^+)}{V} \\ &= S_g^2 g_1 - \frac{S_g \alpha_1}{V} + \frac{1}{V} \frac{\alpha_2 - \alpha_1}{t_2 - t_1}. \end{aligned}$$

Thus, α_2 is uniquely identifiable if V, S_g, g_1 and α_1 are known:

$$\alpha_2 = V(t_2 - t_1)\left[\ddot{y}(t_1^+) - S_g^2 g_1 + \frac{S_g \alpha_1}{V}\right] + \alpha_1.$$

A similar result is obtained for α_i when considering t_i^+, for $i = 2, \ldots, n$.

Since this model is system unidentifiable, we need to include more biological information. A natural choice is to fix V to an accepted value for glycerol distribution volume. In this case, all other parameters are uniquely identifiable.

4.3 PARAMETER ESTIMATION

This section focuses on the system describing glycerol concentrations, $g(t)$, over time:

$$\frac{dg(t)}{dt} = -S_g g(t) + \frac{Ra(t)}{V}, \qquad g(0) = g_b \tag{4.8}$$

with

$$Ra(t) = \begin{cases} \alpha_{i-1} + \frac{\alpha_i - \alpha_{i-1}}{t_i - t_{i-1}}(t - t_{i-1}), & t_{i-1} \leq t \leq t_i, i = 1, \ldots, n \\ 0, & \text{otherwise} \end{cases}. \tag{4.9}$$

We begin by describing the algorithm used to estimate the model parameters, and end by applying this numerical implementation to 3 obese control subjects and 3 obese PCOS subjects.

4.3.1 ALGORITHM

As described in §4.2, we need to fix V so all the other parameters $(S_g, \alpha_i, i = 0, \ldots, n)$ are uniquely identifiable. In the case of glucose, V is complex and subject-specific since the distribution volume for glucose includes plasma and cerebrospinal fluid since glucose can be absorbed directly from the brain. This is why we needed to estimate glucose V in a reference model. However, glycerol does not behave the same way as glucose. Glycerol distribution volume is assumed to be the plasma volume (a commonly known quantity; 300 ml/kg or 5% body weight) [6]. Once glycerol enters a space other than plasma, it converts to a substance other than glycerol; therefore we do not need to concern ourselves with those volumes. For this section, we take $V = 0.3$ L/kg. In this situation, there is no need for Steele's Ra since we do not use a reference model. Since we fix V, we are able to directly estimate S_g and $\alpha_i, i = 0, \ldots, n$, and obtain a model-dependent measure of glycerol Ra.

The algorithm is as follows:

Load data

for $i = 1 : k$

Set random value for parameters

S_g *= rand();* $\vec{\alpha}$ *= rand(1,breakpoints); (V fixed to 0.3)*

Use fmincon to find the minimum of the error function subject to defined constraints

fmincon uses ode45 with given input parameters to calculate error

The error function uses the 2-norm

Constraint: nonnegative parameters

Save parameters that minimize the error function for this k

end

Determine which parameters correspond to the global minimum for the error function

Use ode45 to generate the numerical solution with this parameter set and plot the results

We use the minimizer **fmincon** because we want to ensure that our parameters are nonnegative. However, a limitation of **fmincon** is the possibility that it may find a local minimum instead of the global minimum. To account for this limitation, we loop over the main code a predetermined number of times, k, and we take the global minimum to be the minimum of the k local minima. Since each iteration involves new initial parameter values, this approach should, for sufficiently large k, yield the global minimum. For the results that follow, $k = 30$.

4.3.2 RESULTS

Here we implement the numerical algorithm and estimate $S_g, \alpha_i, i = 0, \ldots, n$ for each subject. We define $V = 0.3$ L/kg and $pV = 0.027$ L/kg for each subject. Although samples were collected at $t = 10, 20$, these samples were only analyzed for isotope concentrations; therefore, glycerol measurements are not available for these time points. All glycerol data presented here was analyzed by the Clinical and Translational Research Center (CTRC).

Subject 1: Subject 1 is an obese control. The parameter results are shown in Table 19 and the numerical solution for glycerol and Ra are shown in Figure 4.2. The glycerol model provides a good fit to the data.

Table 19: Subject 1 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	9.396899401999999e+00	S_g	2.049025807258091e-01
45	3.735668818153647e+00		
75	4.079460223551954e+00		
105	3.371131955031809e+00		
135	3.723879178467989e+00		
180	3.194882437553595e+00		
240	9.167409373680028e+00		
Error	13.1602		
Time (s)	375.178260		

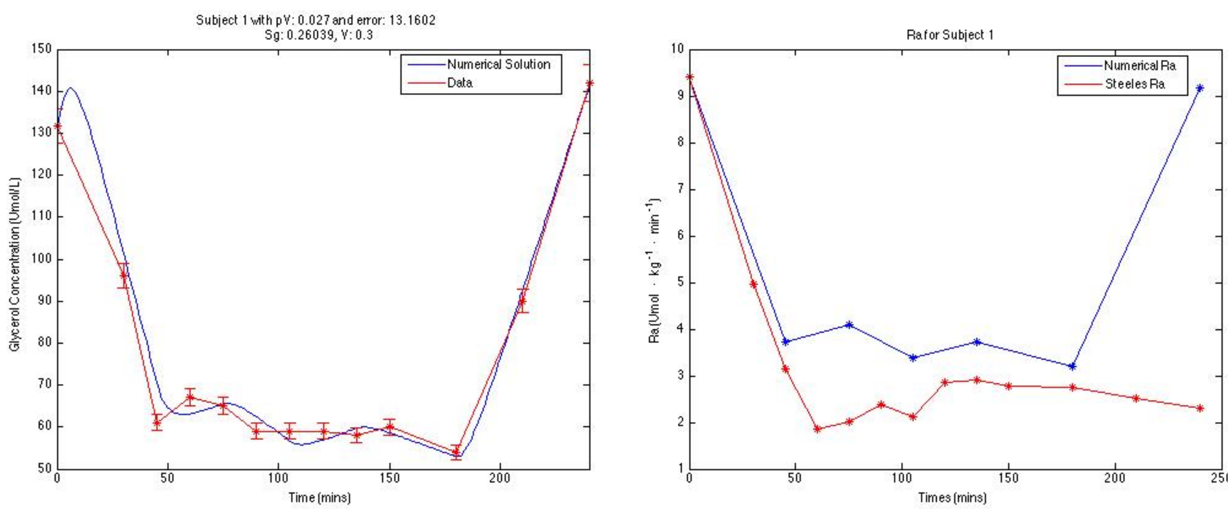

Figure 4.2: Subject 1 Glycerol Numerical Solution and Numerical Ra with $pV = 0.027$, $V = 0.3$

Subject 2: Subject 2 is also an obese control. The parameter results for this subject are shown in Table 20. The numerical solution and numerical Ra are in Figure 4.3. As we can see, the proposed glycerol model produces a good fit to the data.

Subject 3: Subject 3 is the last obese control subject. The parameter results for this subject are shown in Table 21. The numerical solution and numerical Ra are shown in Figure 4.3.

Table 20: Subject 2 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	*Ra* Value		
0	1.392040835200000e+01	S_g	2.706715819187628e-01
45	5.757207006242418e+00		
75	4.970499442050730e+00		
105	5.207980548192749e+00		
135	5.429906748087141e+00		
180	6.393776794706075e+00		
240	6.856238160259190e+00		
Error	6.5544		
Time (s)	458.938907		

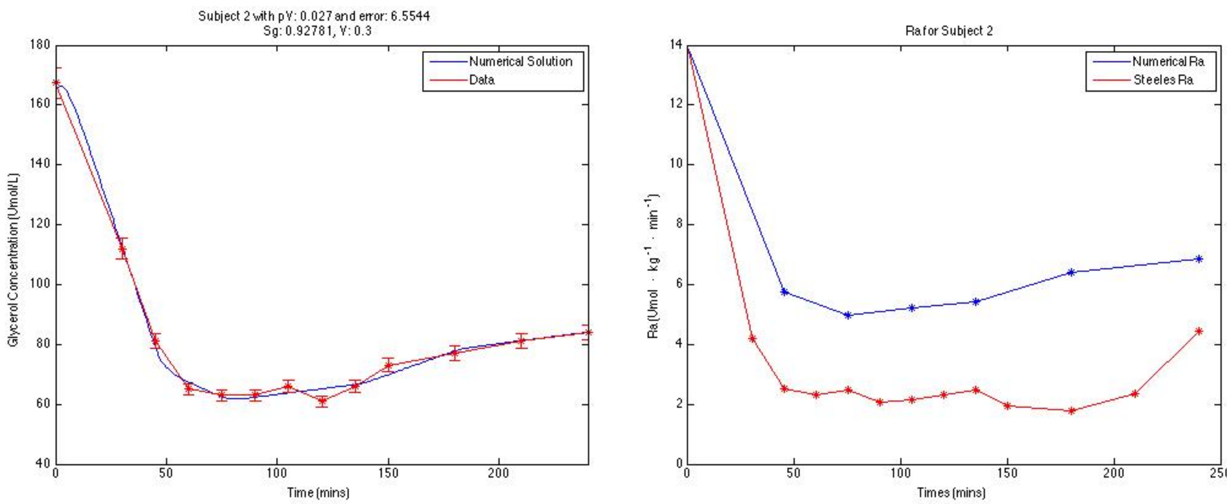

Figure 4.3: Subject 2 Glycerol Numerical Solution and Numerical *Ra* with $pV = 0.027$, $V = 0.3$

Table 21: Subject 3 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	*Ra* Value		
0	6.836881603499894e+00	S_g	1.556448594390546e-01
45	4.276806637834708e+00		
75	2.347142670452072e+00		
105	2.466962891244804e+00		
135	4.530487405045270e+00		
180	2.404923447417271e+00		
240	3.050631203023155e+00		
Error	23.1649		
Time (s)	546.377182		

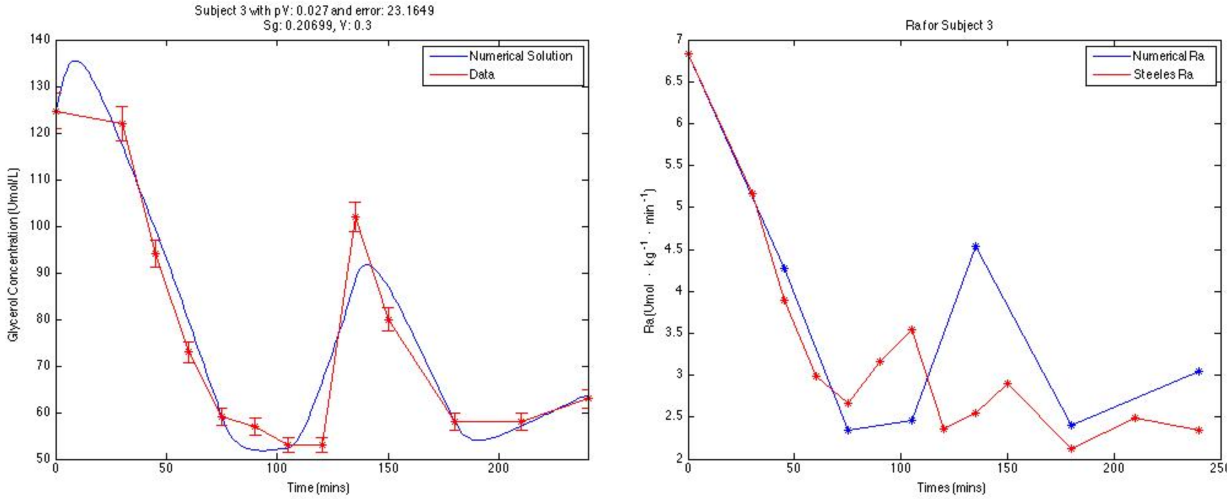

Figure 4.4: Subject 3 Glycerol Numerical Solution and Numerical Ra with $pV = 0.027$, $V = 0.3$

Subject 4: Subject 4 is in the Obese PCOS group. The parameter results for this subject are shown in Table 22. The numerical solution and numerical Ra are shown in Figure 4.5. The numerical solution is a good fit to the data. Additionally, we show that this model can calculate a model-dependent measure of glycerol Ra since numerical Ra agrees with Steele's Ra.

Table 22: Subject 4 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	2.872804382500000e+00	S_g	1.201877212979154e-01
45	1.707179835516597e+00		
75	1.995138387426920e+00		
105	1.721160341480962e+00		
135	1.729460746133123e+00		
180	2.136040578642838e+00		
240	1.955810137111221e+00		
Error	7.4607		
Time (s)	527.167100		

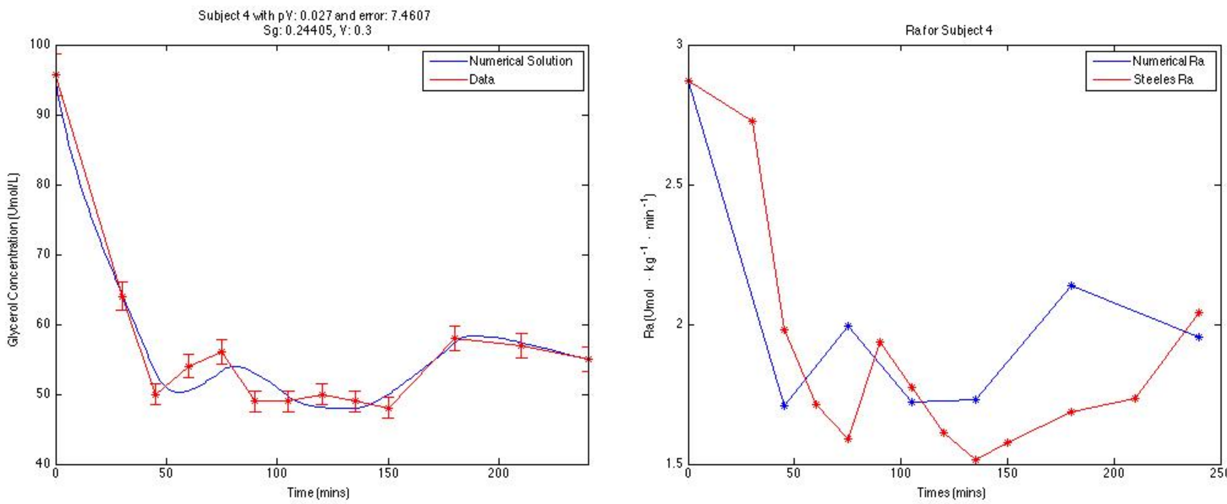

Figure 4.5: Subject 4 Glycerol Numerical Solution and Numerical Ra with $pV = 0.027$, $V = 0.3$

Subject 5: Subject 5 is an Obese PCOS subject. The parameter results for this subject are shown in Table 23. The numerical solution and numerical Ra are shown in Figure 4.6.

Table 23: Subject 5 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	2.836762081497499e+01	S_g	2.532685384619465e-01
45	5.545135377257649e+00		
75	6.919268178787366e+00		
105	6.535630498156975e+00		
135	4.171470512447723e+00		
180	7.110075211908224e+00		
240	2.083775650645141e+01		
Error	49.2768		
Time (s)	451.351188		

Subject 6: Subject 6 is the last subject in the Obese PCOS category. The parameter results for this subject are shown in Table 24. The numerical solution and numerical Ra are shown in Figure 4.7.

Summary: We had proposed using S_g as a measure for adipose IR. Table 25 summarizes the results for the parameter S_g. With a small sample size, we cannot determine if this parameter is a useful measure for quantifying adipose IR.

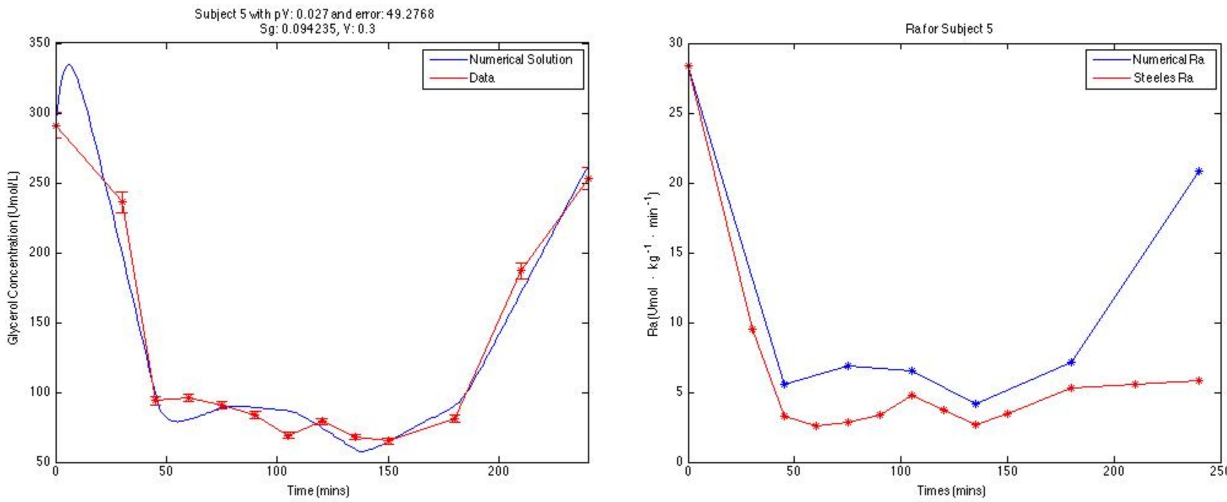

Figure 4.6: Subject 5 Glycerol Numerical Solution and Numerical Ra with $pV = 0.027$, $V = 0.3$

Table 24: Subject 6 Glycerol Model Parameter Results

Parameter	Value	Parameter	Value
Ra Time	Ra Value		
0	5.813379744340184e+00	S_g	1.860882970940433e-01
45	2.891951589643761e+00		
75	3.773447541365297e+00		
105	3.762492413977251e+00		
135	4.116446202268625e+00		
180	2.836962594753611e+00		
240	6.795275248171913e+00		
Error			
Time (s)	376.553716		

Table 25: S_g Results

Subject #	S_g
1	2.049025807258091e-01
2	2.706715819187628e-01
3	1.556448594390546e-01
4	1.201877212979154e-01
5	2.532685384619465e-01
6	1.860882970940433e-01
Mean	1.984605964895886e-01
Control	2.104063406945421e-01
PCOS	1.865148522846351e-01
t-test	
h	0
p	0.6630

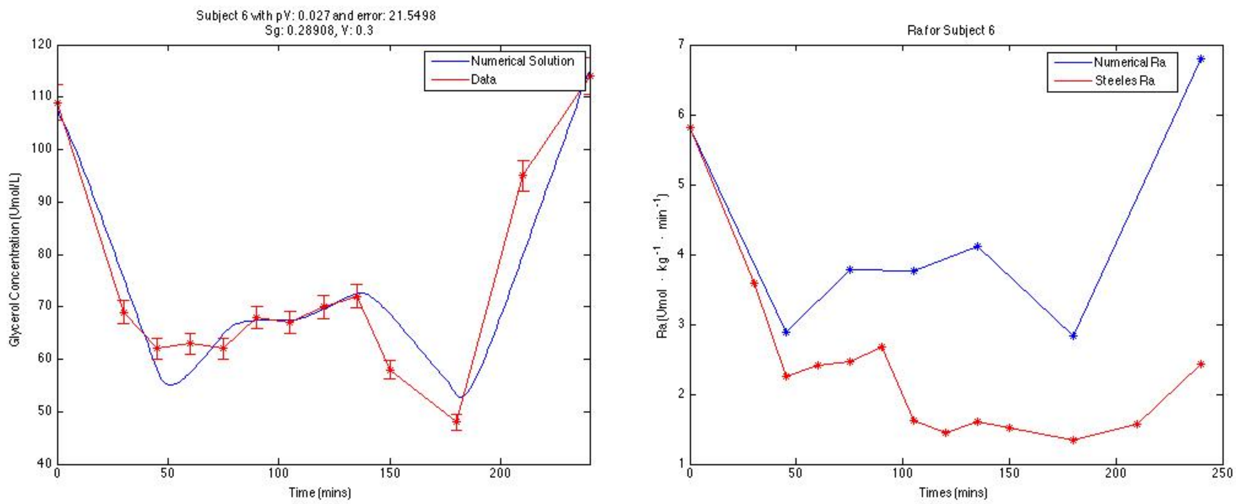

Figure 4.7: Subject 6 Glycerol Numerical Solution and Numerical Ra with $pV = 0.027$, $V = 0.3$

4.4 OTHER METRICS

In this section, we will explore other metrics including time to 50% suppression of lipolysis, glycerol concentration at 50% suppression of lipolysis, time to minimum glycerol concentration, minimum glycerol concentration, average basal concentration, and area bounded between the numerical solution and the baseline concentration. These values will also be compared with insulin concentrations. We will use the numerical solutions found in §4.3.2 for each subject. The numerical results involving these metrics for each subject are presented in Figure 4.8.

The results from MATLAB's built-in function **ttest2** show that with this small population, there are no conclusive results. Time to 50% Suppression and Time to Peak Insulin may be metrics of interest when additional data becomes available. These two metrics are compared in Figure 4.9. It is possible that Subject 2 is an outlier in the Obese Control group, and there may be a significant difference between these two groups in regards to these two metrics. Obese PCOS may have lower Time to Peak Insulin concentration and lower Time to 50% Suppression compared to their Obese Control counterparts.

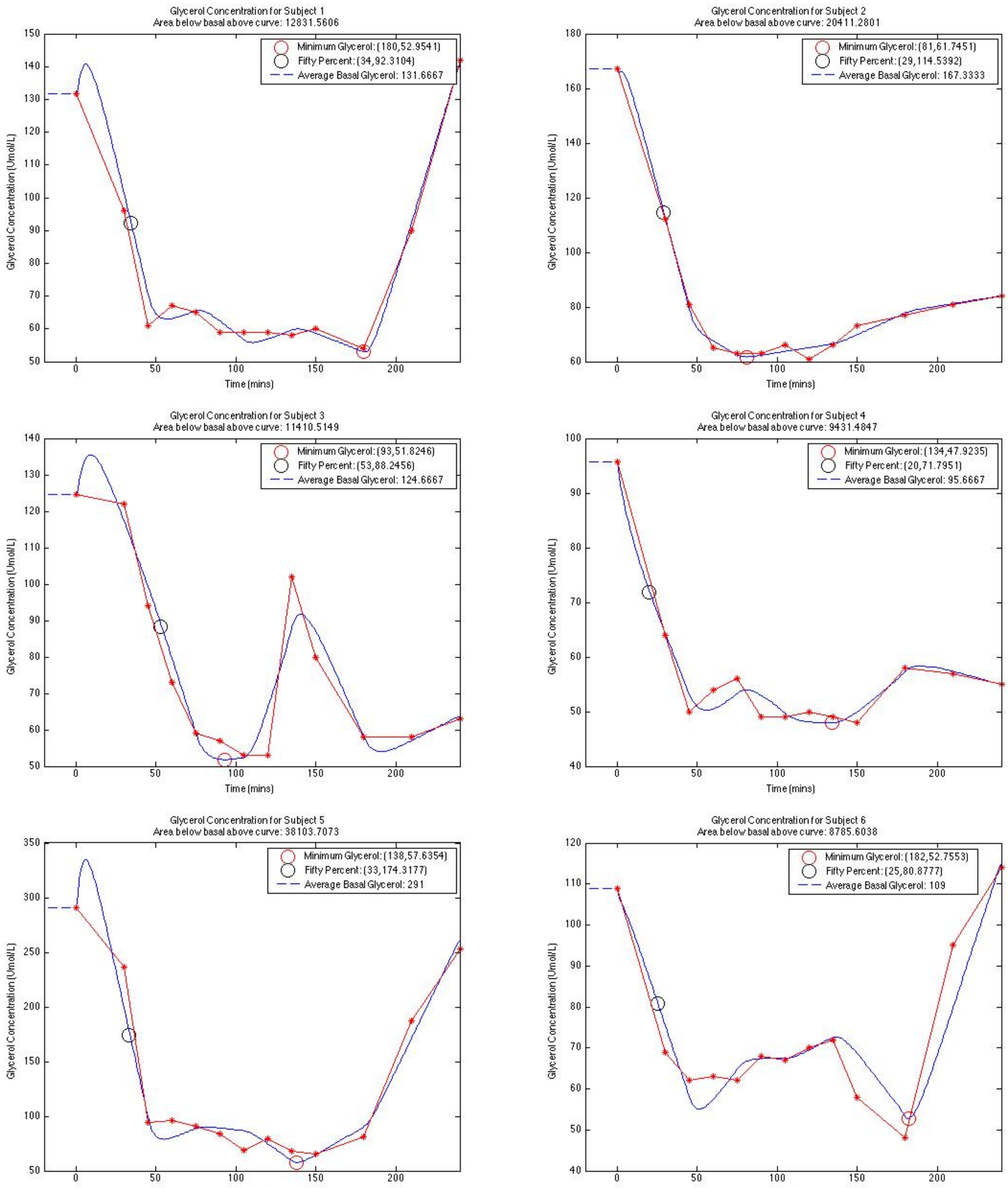

Figure 4.8: Glycerol Numerical Solution for All Subjects with Other Metrics Indicated

Table 26: All Subject Glycerol Other Metics

Subject #	Average Basal Glycerol (Umol/L)	Time to Minimum Glycerol (mins)	Minimum Glycerol Concentration (Umol/L)	Time to 50% Suppression (min)	50% Suppression Concentration (Umol/L)	Time to Peak Insulin (min)	Peak Insulin Concentration (μ U/ml)	Area Above Numerical Solution Under Basal (mins $\cdot$ Umol/L)
1	131.6667	180	52.9541	34	92.3104	120	173	12831.5606
2	167.3333	81	61.7451	29	114.5392	30	381	20411.2801
3	124.6667	93	51.8246	53	88.2456	120	510	11410.5149
4	95.6667	134	47.9235	20	71.7951	45	238	9431.4847
5	291	138	57.6354	33	174.3177	30	257	38103.7073
6	109	182	52.7553	25	80.8777	45	608	8785.6038
Mean	153.2221	134.6667	54.1397	32.3333	103.6809	65	361.1667	16829.0252
· Control	141.2220	118	55.5079	38.6667	98.3651	90	354.6667	14884.4518
· PCOS	165.2222	151.3333	52.7714	26	108.9968	40	367.6667	18773.5986
$t-$test ($\alpha = 0.05\%$)								
h	0	0	0	0	0	0	0	0
p	0.72820	0.3921	0.5508	0.1987	0.7686	0.1755	0.9373	0.7188

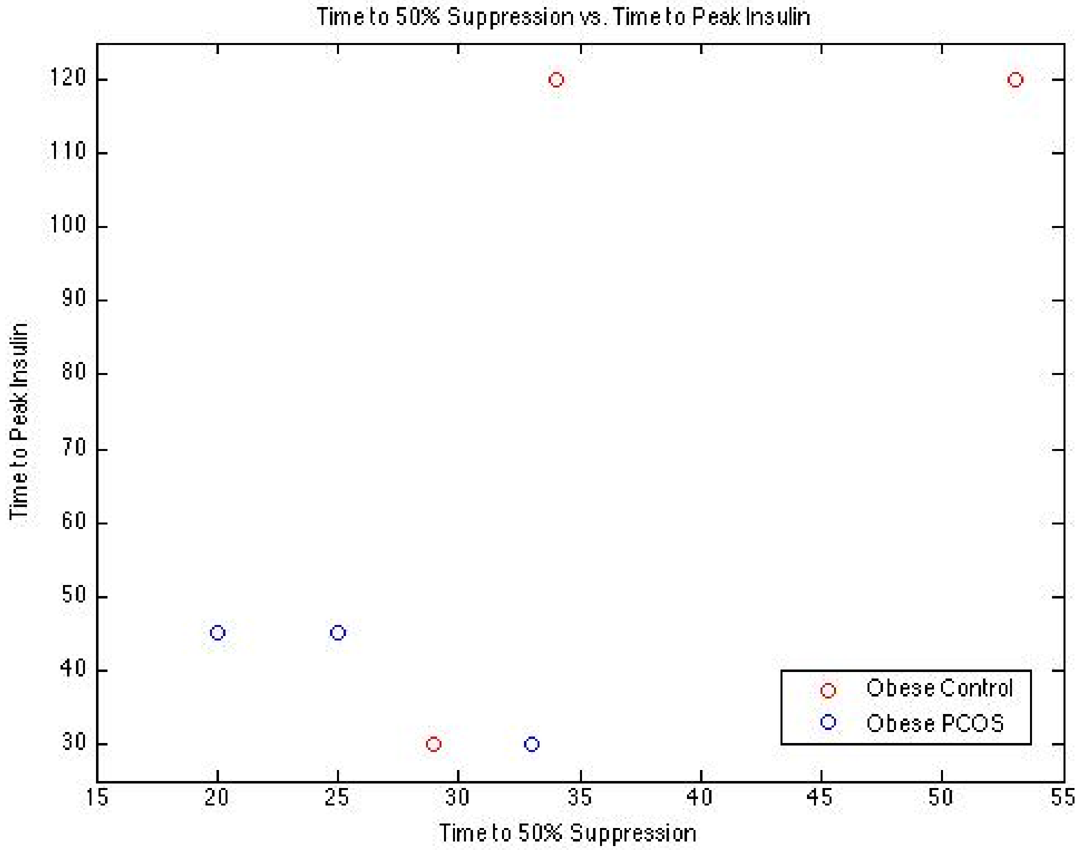

Figure 4.9: Comparison of Time to 50% Suppression and Time to Peak Insulin

Since the proposed glycerol model produces a model-dependent measure of glycerol *Ra*, considering other metrics related to *Ra* may be of interest. A summary of metrics concerning numerical *Ra* from the glycerol model and Steele's *Ra* are shown in Table 27. There are no metrics that show a significant difference between the Obese Control and Obese PCOS groups. However, we may use this information to help validate the glycerol model by comparing numerical *Ra* to Steele's *Ra*. There is strong agreement between Basal Numerical *Ra* and Basal Steele's *Ra*. In general, Minimum Numerical *Ra* is larger than Minimum Steele's *Ra*. This discrepancy may be from the generalization for the distribution volume V. The scale for V is correct, but future work considering a range for V would be interesting ($1.2 \leq V \leq 3.2$, for example).

Table 27: Other Metrics using Ra

Subject #	Basal Numerical Ra	Basal Steele's Ra	Time to Minimum Numerical Ra	Minimum Numerical Ra	Time to Minimum Steele's Ra	Minimum Steele's Ra
1	9.3969	9.3969	180	3.1949	60	1.8603
2	13.9204	9.2452	75	4.9705	180	1.7707
3	6.8369	6.8369	75	2.3471	180	2.1257
4	1.7072	2.8728	45	1.7072	135	1.5155
5	28.3676	28.3676	135	4.1715	60	2.5792
6	5.8134	5.8134	180	2.8370	180	1.3400
$t-$test ($\alpha = 0.05\%$)						
h	0	0	0	0	0	0
p	0.8339	0.6585	0.8593	0.5993	0.7918	0.8025

4.5 GLYCEROL CONCLUSION

This chapter focused on a novel glycerol model to describe glycerol dynamics during an Oral Glucose Tolerance Test (OGTT). We started by investigating the identifiability of the proposed model; the model was system unidentifiable (§4.2). This analysis lead to a numerical approach that was applied to 6 obese adolescent females (§4.3). We were unable to find a definitive measure for distinguishing adipose IR in PCOS (§4.3.2, 4.4). However, the glycerol model proposed in this chapter may be useful to future adipose IR research during an OGTT. For example, we have shown that this model fits the data to 6 subjects and produces a model-dependent measure for Ra. The disadvantage of using Steele's Ra is that to calculate n Ra values, it is necessary to have $n+1$ glycerol concentration data points. Using the proposed glycerol model, it may be possible to use fewer data points to fit the model but still have the same number of data points for numerical Ra. This would mean fewer blood samples needed to determine glycerol dynamics. This frees blood samples for other substrates, or even reduces the impact of the study on the subject by requiring fewer blood samples in general. To summarize, this chapter aids in the understanding of glycerol dynamics after a meal.

CHAPTER 5
CONCLUSION

The goal of this project was to determine the contributions of hepatic and adipose tissue to IR in PCOS using a modified OGTT protocol. For hepatic IR, we used OMM* to calculate S_I^* and quantify hepatic IR. We found that our subjects (Obese Control and Obese PCOS) have low S_I^* values, which indicates hepatic IR may be implicated in obese adolescent females. For adipose IR, we developed a novel model for glycerol dynamics during an OGTT. We fit the model to all subjects and calculated a model-dependent rate of appearance for glycerol. Unfortunately, additional work with a larger sample size is needed to determine if hepatic or adipose IR is present in PCOS. This work contributes to an increased understanding of hepatic and adipose IR, and further work may aid in targeted therapeutics for girls and women with PCOS.

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A MATLAB CODE

This appendix contains MATLAB code for the glucose and glycerol simulations.

A.1 GLUCOSE CODE

The following MATLAB code was used for the glucose simulations.

RM_meal.m

```
% Glucose RM star

function [theta,ESS] = RM_meal(sub_number)

tic

% Set global variables
global INITIALCONDITION times glucose insulin Ra t_vals Ra_fit I

% Load glucose data
% For this simulation, glucose = G_meal
% [times,g_tot,g_meal,insulin,Ra_tot,Ra_endo,Ra_exo]
%[times,g_tot,glucose,insulin,Ra_tot,Ra_endo,Ra_exo] =
    average_glucose_data;
[times,g_tot,glucose,insulin,Ra_tot,Ra_endo,Ra_exo] = glucose_data(
    sub_number);

% Average basal values for initial time point
g_basal = mean([g_tot(1:4)]);
g_tot(4) = g_basal;
gmeal_basal = mean([glucose(1:3)]);
glucose(3) = gmeal_basal;
```

```
insulin_basal = mean([insulin(1:3)]);
insulin(3) = insulin_basal;

% Truncate to start at zero
times = times(4:end);
glucose = glucose(3:end); % no value for -30
insulin = insulin(3:end);
Ra = Ra_exo(3:end);

% Ra = Ra_exo;
% glucose = g_meal;

Ra_fit = pchip(times,Ra);
I = pchip(times,insulin);

% Set Parameters and initial conditions
G0 = glucose(1);
X0 = 0;
I0 = insulin(1);
INITIALCONDITION = [G0; X0];

tf = times(end);
dt = 1; %------ NOTE: changing this in ODE solver to be easier
t_vals = 0:dt:tf;

SG = .15;%.1*rand();%0.15;%8.6275074385698500;
p2 = .01;%.01*rand();%0.01;%0.0000000046271000;
p3 = 0.00004;%.001*rand();%0.00004;%0.0003962816091609;
V = .05;%1+rand();%0.05;%0.0014537990080098;


params = [SG  p2  p3  V];
```

```
lb = [0,0,0,0];
ub = [10,10,10,10];

OPTIONS = optimoptions('fmincon','Algorithm','interior-point',...
    'PlotFcns',{@optimplotx,...
    @optimplotfval});
[theta,ESS] = fmincon(@error_glu1,params,[],[],[],[],lb,ub,[],OPTIONS)

% Integrate the system using ode45.m.
ode_options = odeset('RelTol',1e-6);
[t,Y] = ode45(@Glucose_rhs,t_vals,INITIALCONDITION,ode_options,theta);

e = 0.02*glucose;

% Plot the results.
figure;clf;
plot(t,Y(:,1),times,glucose,'r*')%,'x','linewidth',3)
hold on
errorbar(times,glucose,e,'r')
title({['Meal Glucose RM* for Subject ' num2str(sub_number)];['Error :'
    num2str(ESS)]} )
%title({['Meal Glucose RM* for Cobelli Subject '];['Error :' num2str(
    ESS)]} )
xlabel('Time (mins)')
ylabel('Glucose Concentration')

figure;clf;
plot(t,Y(:,2))
title(['X*(t) RM* Meal Glucose Subject ' num2str(sub_number)])
%title(['X*(t) RM* Meal Glucose for Cobelli Subject '])
xlabel('Time (mins)')
```

```
ylabel('X*')

toc
end

function dy = Glucose_rhs(t,y,pars)
% pars = p1=SG, p2, p3, V
% y = G, X
global insulin I Ra_fit

dy = [-(pars(1) + y(2))*y(1) + ppval(Ra_fit,t)/pars(4);
        -pars(2)*y(2) + pars(3)*(ppval(I,t) - insulin(1))];

end


function ess = error_glu1(pars)
    global times glucose t_vals INITIALCONDITION

    % Solve the ode with the incoming pars
    ode_options = odeset('RelTol',1e-6);
    [t,y] = ode45(@Glucose_rhs,times,INITIALCONDITION,ode_options,pars)
        ;
    % Note: we can use t_vals here, but it increases computation time
    % Also, we need to use the appropriate diff calculation

    % Initialize error
    ess = 0;

    % This for loop should work with nonuniform measurement times AND
    % t_vals is 0:k:times(end)
%       for i = 1:length(glucose)
```

```
%           for j = 1:length(y)
%               if t(j) == times(i)
%                   diff(i) = glucose(i) - y(j);
%               end
%           end
%       end

    % Below for loop is when t_vals is 0:1:times(end)
%       for i = 1:length(glucose)
%           diff(i) = glucose(i)-y(times(i)+1,1);
%       end

    % This is for the ode solver above using times
    diff = glucose' - y(:,1);

    ess = norm(diff,2);

end
```

RM_total.m

```
% This code does RM for total glucose

function RM_total(sub_number)
tic
% Set Global Variables
global INITIALCONDITION t_vals times glucose Ra_fit I_fit insulin

% Load data from glucose_data file
% [times,g_tot,g_meal,insulin,Ra_tot,Ra_endo,Ra_exo]
[times,glucose,glucose_meal,insulin,Ra_tot,~,Ra_exo] = glucose_data(sub_number);

```

```
% Average basal values for initial time point
g_basal = mean([glucose(1:4)]);
glucose(4) = g_basal;
gmeal_basal = mean([glucose_meal(1:3)]);
glucose_meal(3) = gmeal_basal;
insulin_basal = mean([insulin(1:3)]);
insulin(3) = insulin_basal;

% Change data to start at zero      % corresponding time points:
times = times(4:end);               % times = -30,-20-10,0
glucose = glucose(4:end);           % total glucose = -30,-20,-10,0
glucose_meal = glucose_meal(3:end); % meal glucose = -20,-10,0
insulin = insulin(3:end);           % insulin = -20,-10,0
Ra_tot = Ra_tot(3:end);             % Ra_tot = -20,-10,0
Ra_exo = Ra_exo(3:end);             % Ra_exo = -20,-10,0 (NOTE: values
    =0)
% times = times(4:end-7);                 % times = -30,-20-10,0
% glucose = glucose(4:end-7);             % total glucose = -30,-20,-10,0
% glucose_meal = glucose_meal(3:end-7); % meal glucose = -20,-10,0
% insulin = insulin(3:end-7);             % insulin = -20,-10,0
% Ra_tot = Ra_tot(3:end-7);               % Ra_tot = -20,-10,0
% Ra_exo = Ra_exo(3:end-7);               % Ra_exo = -20,-10,0 (NOTE:
    values=0)

% Set initial condition
G0 = glucose(1);          % Total glucose (G)
X0 = 0;                   % Insulin action total (X)
INITIALCONDITION = [G0; X0];

% Set grid size for ODE solver
tf = times(end);          % Final time
dt = 1;                   % Step size (NOTE: CHANGE DIFF CALC BELOW)
```

```
                                % Set to 0 for t_vals = times
%t_vals = times(1):dt:tf; % Grid
t_vals = times;

% Set up interpolated functions for Steeles Ra and insulin - used in solver
Ra_fit = pchip(times,Ra_exo); % pchip(X,Y) provides the piecewise polynomial
I_fit = pchip(times,insulin); % to the values Y at the sites X,
                                % for later use with PPVAL

% Set initial parameter values
% For RM, the parameters are SG, p2, p3, V, since Ra is fixed
% Parameters for G system:
SG = 2.217328845895088e-01 ;%0.0118;%0.15;%.1*rand();
p2 =    2.080971108661180e-09;%0.039;%0.01;%.01*rand();
p3 =  2.095237072512212e-05 ;%0;%0.00004;%.001*rand();
V = 4.067744813643938e-02;%1.6;%.05;%1+rand();

params = [SG, p2, p3, V];

% Set the contraints for fmincon
lb = [0,0,0,0];                       % lower bound is 0
ub = [10,10,10,10];                   % upper bound is +inf

% Set options for fmincon
% Can include: 'Display','iter','TolX',1e-20
% OPTIONS = optimoptions('fmincon','Algorithm','interior-point');

% fmincon - solve for parameters and minimum error
% fmincon(FUN,X0,A,B,Aeq,Beq,LB,UB,NONLCON)
OPTIONS = optimoptions('fmincon','Algorithm','interior-point',...
```

```
    'PlotFcns',{@optimplotx,...
    @optimplotfval});
[theta, ESS] = fmincon(@error_glucose,params,[],[],[],[],lb,ub,[],
    OPTIONS)

% Integrate the system using ode45.m using the new parameters
t_vals2 = times(1):1:times(end);
% ode_options = odeset('RelTol',1e-6);
[t,Y] = ode45(@Glucose_rhs,t_vals2,INITIALCONDITION,[],theta);

% Set error bars
e = 0.02*glucose;

% Plot the results.
figure;clf;
plot(t,Y(:,1),times,glucose,'r*')%,'x','linewidth',3)
hold on
errorbar(times,glucose,e,'r')
%title({['Total Glucose RM for Subject ' num2str(sub_number)];['Total
    Error :' num2str(ESS)]} )
title({['Total Glucose RM for Average Subject '];['Total Error :'
    num2str(ESS)]} )
xlabel('Time (mins)')
ylabel('Glucose Concentration')
legend('Numerical Solution', 'Data')

figure;clf;
plot(t,Y(:,2))
%title(['X(t) RM Total Glucose Subject ' num2str(sub_number)])
title(['X(t) RM Total Glucose for Average Subject '])
xlabel('Time (mins)')
ylabel('X')
```

```
toc
end

function [ESS_total] = error_glucose(pars)
% Collect global variables
global INITIALCONDITION t_vals times glucose

% Set ode options for ode solver
ode_options = odeset('RelTol', 1e-6);
% ode45(ODEFUN,TSPAN,Y0,OPTIONS), add parameters at the end
[t,y] = ode45(@Glucose_rhs,t_vals,INITIALCONDITION,ode_options,pars);

% Initialize error
ESS = 0;

% Calculate difference
%if dt == 1          % When t_vals = 0:1:tf
%      for i = 1:length(glucose)
%            diff(i) = glucose(i) - y(times(i)+1,1);
%            diff_meal(i) = glucose_meal(i) - y(times(i)+1,3);
%      end
%end

% When t_vals = times
diff = glucose' - y(:,1);

ESS_total = norm(diff,2);

end

function dy = Glucose_rhs(t,y,pars)
```

```
% Pars = [SG, p2, p3, V]
% Collect global variables
global glucose Ra_fit I_fit insulin

% NOTE: ppval(PP,XX) returns the value, at the entries of XX

dy = [-(pars(1)+y(2))*y(1) + pars(1)*glucose(1) + ppval(Ra_fit,t)/pars(4);
      % ^ total glucose
      -pars(2)*y(2) + pars(3)*(ppval(I_fit,t) - insulin(1))];
      % ^ total insulin action

end
```

FM_together.m

```
function FM_together
% This function should produce the final solution for the glucose code

tic % time the code

% Set global variables
global t_vals INITIALCONDITION g_meal times Ra_tvals V V_m I_fit I0 ...
    Total_ingested g_tot SG SG_m Ra_exo

% Print many decimals
format long e

% Load the data from glucose_data.m
[times,g_tot,g_meal,insulin,Ra_tot,Ra_endo,Ra_exo] = average_glucose_data;

% Average basal values for initial time point
```

```
g_basal = mean([g_tot(1:4)]);
g_tot(4) = g_basal;
gmeal_basal = mean([g_meal(1:3)]);
g_meal(3) = gmeal_basal;
insulin_basal = mean([insulin(1:3)]);
insulin(3) = insulin_basal;

% Truncate data to start at time 0
times = times(4:end);
g_tot = g_tot(4:end);
g_meal = g_meal(3:end); % no value for -30 (by design)
insulin = insulin(3:end);
Ra_exo = Ra_exo(3:end);

% Fit the insulin data to use in the ODE function (G_rhs)
I_fit = pchip(times,insulin);

% Total ingested glucose is used in the Ra_constraint
% \int Ra meal = Df, D is ingested glucose, f is absorbed portion
% Change this to fit with the spreadsheet data (glucose tracer data)
% f = .87; % fraction absorbed
% D = 297; % given ingested dose
%f = .87;
D = 78523;
%Total_ingested = f*D;
f = trapz(times,Ra_exo)/D;
Total_ingested = f*D;

% Set initial condition
G0 = g_tot(1);
X0 = 0;
G0_m = g_meal(1);
```

```
X0_m = 0;
I0 = insulin(1);
INITIALCONDITION = [G0; X0; G0_m; X0_m];

% Set time for ode solver (final solution image)
ti = times(1);
tf = times(end);
dt = 1;
t_vals = [ti:dt:tf];

% Set fixed parameter value
V = 0.0507768550758991;
V_m = 0.0248750670569493;
SG = 0.2070696394719850;
SG_m = 0.4712102692891860;

% Initialize guess for parameters
Ra_tvals = zeros(1,8);
for i = 0:7
    Ra_tvals(i+1) = times(2*i+1);
end
Ra = ones(1,length(Ra_tvals));

p2 = 0.0000000036873346;
p3 = 0.0000150477717492;

params_tot = [Ra p2 p3];

p2_m = 0.0000000002755050;
p3_m = 0.0000207270554656;
params_m = [p2_m p3_m];

```

```
params = [params_tot params_m];

% Set lower and upper bounds on the parameter values (nonnegative)
lb = zeros(1,length(params));
ub = [5*ones(1,length(Ra)) .5 .5 .5 .5];

% Use fmincon to find the parameter values
% fmincon(function,initial params,A,b,Aeq,beq,lb,ub,nonlcon)
% min params subject to: Ax <= b, Aeqx = beq, lb <= x <= ub, nonlinear(
    x)
OPTIONS = optimoptions('fmincon','Algorithm','interior-point',...
    'PlotFcns',{@optimplotx,...
    @optimplotfval});
    %'TolX',1e-3,...
[theta,ESS] = fmincon(@error_fun,params,[],[],[],[],lb,ub,
    @Ra_constraint,OPTIONS)

% Solve the ode for plotting using a fine grid (t_vals) and the
    minimized
% parameter values
t_vals2 = [0:1:times(end)];
[t,y] = ode45(@G_rhs,t_vals2,INITIALCONDITION,[],theta);

for i = 1:length(times)
    diff_total(i) = abs(g_tot(i) - y(times(i)+1,1));
    diff_meal(i) = abs(g_meal(i) - y(times(i)+1,3));
end
ess_total = norm(diff_total,2);
ess_meal = norm(diff_meal,2);


% Define the error bars for plotting
```

```
e = 0.02*g_tot;
e_meal = 0.02*g_meal;

% Plot the numerical solution and the data
figure; clf;
plot(t,y(:,1),'b',times,g_tot,'r*')
hold on
plot(times,g_tot,'r')
hold on
errorbar(times,g_tot,e,'r')
title({'Average Total Glucose',['Error: ' num2str(ess_total)]})
xlabel('Time (mins)')
ylabel('Glucose Concentration')
legend('Numerical Solution', 'Data')

figure; clf;
plot(t,y(:,3),'b',times,g_meal,'r*')
hold on
plot(times,g_meal,'r')
hold on
errorbar(times,g_meal,e_meal,'r')
title({'Average Meal Glucose', ['Error: ' num2str(ess_meal)]})
xlabel('Time (mins)')
ylabel('Glucose Concentration')
legend('Numerical Solution', 'Data')

% Plot numerical Ra and Steeles Ra
figure; clf;
% plot(Ra_tvals,theta(1:end-6),'b')
plot(Ra_tvals,theta(1:end-4),'b')
hold on
plot(times,Ra_exo,'r')
```

```
title('Average Subject Ra Values')
xlabel('Time (mins)')
ylabel('Meal glucose Ra')
legend('Numercal Ra', 'Steeles Ra')

toc
end

function er = error_fun(pars)
% This function is the error function that fmincon minimizes
% it needs to return a scalar value to minimize (potential issue when
% trying to minimize both OMM and OMM* at the same time)

% Collect global variables
global INITIALCONDITION g_meal times t_vals g_tot

% Solve the ode with the incoming parameters (pars)
[t,y] = ode45(@G_rhs,times,INITIALCONDITION,[],pars);
% NOTE: we can use a fine grid here, as in t_vals instead of times, but
% this adds to computation time. Also, ode45 uses a time adaptive
% Runge-Kutta method. This means that the time vector entered into the
    ode
% solver returns the solution at those time points, but it sends
    different
% time values into the ODE for its own stability/convergence

% This is the error calculation at each time point when we use times in
    the
% solver
diff = abs(g_tot'-y(:,1));
diff_meal = abs(g_meal'-y(:,3));

```

```
% We need to use a new method if we use t_vals (which is 0:1:times(end)
    )
% for i = 1:length(times)
%      diff(i) = abs(g_meal(i) - y(times(i)+1,1));
% end

% Calculate the error

%%%%%%% weight by max glucose? diff/max
weighted_diff = diff/max(g_tot);
weighted_diff_meal = diff_meal/max(g_meal);

error_vec = [weighted_diff, weighted_diff_meal];
er = norm(error_vec,2);

end

function dy = G_rhs(t,y,pars)
% This is the rhs of the ode - we use it in ode45
% The incoming parameter vector is
% pars = [RA SG P2 P3 SG_m P2_m P3_m]

% Collect global variables
global Ra_tvals V V_m I_fit I0 g_tot SG SG_m

Ra = 0;

% Idea: loop over the Ra_tvals to find where t is
% We need Ra_tvals(i) <= t <= Ra_tvals(i+1) for our Ra definition
for i = 1:length(Ra_tvals)-1
    if ((t<Ra_tvals(i+1)) && (Ra_tvals(i)<t))
        alpha_1 = pars(i);
```

```
            alpha_2 = pars(i+1);
            t_diff = Ra_tvals(i+1)-Ra_tvals(i);
            Ra = alpha_1 + ((alpha_2 - alpha_1)/(t_diff))*(t - Ra_tvals(i));
            break; % we found t; stop looking
        end
    end

        It = ppval(I_fit,t);

        dy = [-(SG + y(2))*y(1) + SG*g_tot(1)+ Ra/V;
                %^ total glucose (G)
            -pars(end-3)*y(2) + pars(end-2)*(It - I0);
                %^ total insulin action (X)
            -(SG_m + y(4))*y(3) + Ra/V_m;
                %^ meal glucose (G_meal)
            -pars(end-1)*y(4) + pars(end)*(It - I0)];
                %^ insulin action on disposal (X*)

end

function [C,Ceq] = Ra_constraint(pars)
% This function is used to ensure the integral of Ra meal is not larger
% than the total ingested glucose

% C(x) <= 0
% Ceq(x) = 0

% Load global vars
global Total_ingested times Ra_tvals Ra_exo

Ceq = pars(1) - 0;
```

```
% For convenience, set the Ra parameter vector
total_Ra_vec = [pars(1:end-4)];

% Use the trapezoid rule to calculate integral
param_Ra_area = trapz(Ra_tvals,total_Ra_vec);

% int Ra(params) - total_ingested <= 0
C = param_Ra_area - Total_ingested;

end
```

A.2 GLYCEROL CODE

The following MATLAB code was used for the glycerol simulations.

Glycerol_FM.m

```
% Glycerol Code

function Glycerol_FM
tic

% Set global variables
global times glycerol tspan V t_vals  Ra_basal sub_number pV ...
    INITIALCONDITION

sub_number = 0; % this is used as a cosmetic value for plotting (0 =
    average subject)
pV = 0.027;

V = 0.3;
% Melanie medical text book: V = 5% of body weight --- units are weird
```

```
% want dl/kg for V
%                   old pV        new pV
%sub1 = 74.4       pV: 0.182
%sub2 = 80         pV: 0.175      0.622
%sub3 = 69.3       pV: 0.038
%sub4 = 96.8       pV: 0.067
%sub5 = 92.6       pV: 0.032
%sub6 = 115.3      pV: 0.041
% Additional source: 300 ml/kg

% Load data
% Average Subject
times = [-20,-10,0,30,45,60,75,90,105,120,135,150,180,210,240];
glycerol = [179.3333333,145.6666667,134.6666667,116.5,73.66666667,...
    69.66666667,66,63.33333333,60.5,62,69.16666667,64,62.66666667,...
    94.66666667,118.5];
Ra = [7.738980174,6.319963576,4.148551232,2.695760539,2.202571362,...
    2.258438538,2.549411835,2.322529074,2.168087954,2.135812213,...
    2.135818056,2.048716013,2.258507933,2.789693324];

% Average basal values for initial time point
g_basal = mean([glycerol(1:3)]);
glycerol(3) = g_basal;
Ra_basal = mean([Ra(1:2)]);
Ra(2) = Ra_basal;

% Truncate data to start at 0
times = times(3:end);
glycerol = glycerol(3:end);
Ra = Ra(2:end);

INITIALCONDITION = [glycerol(1)];
```

```
% Set interval for ODE solver
tspan = [times(1):1:times(end)];

% Set breakpoints for numerical Ra
t_vals = zeros(1,floor(length(times)/2))
floor(length(times)/2)
for i = 0:floor(length(times)/2)
    t_vals(i+1) = times(2*i+1);
end
t_vals
%t_vals = times;

% Set initial parameter values
for i = 1:40
Sg = rand(); % rand() is uniformly distributed between 0-1
alpha = rand(1,length(t_vals));%min(Ra)*ones(1,length(t_vals));

pars = [Sg, alpha];

lb = [0,(min(Ra)-1)*ones(1,length(alpha))];
ub = [1,max(Ra),(max(Ra))*ones(1,length(alpha)-1)];
OPTIONS = optimoptions('fmincon','Algorithm','interior-point',...
    'PlotFcns',{@optimplotx,...
    @optimplotfval});
[theta,ess(i)] = fmincon(@error_gly2,pars,[],[],[],[],lb,ub,
    @nonlinfunction,OPTIONS);

pars_min(:,i) = theta;
end

% Determine the minimum error and the location in the ess vector
```

```
[m,n] = min(ess);
pars_min = pars_min(:,n)

% Save the new calculated Ra. NOTE that Ra = alpha_i-1 + frac{alpha_i -
% alpha_i-1}{t_i - t_i-1}(t - t_i-1) but when we plug in t_i-1, we get Ra =
% alpha_i-1 so we exploit that here
for i = 2:length(pars_min)%-2
    Ra_new(i-1) = pars_min(i);
end


% Plot Ra with Steele's for visual comparison
figure;clf;
plot(t_vals,Ra_new,'b',times,Ra,'r')
hold on
plot(t_vals,Ra_new,'b*',times,Ra,'r*')
if sub_number == 0
    title('Ra for Average Subject ')
else
    title(['Ra for Subject ' num2str(sub_number)])
end
xlabel('Times (mins)')
ylabel('Ra (Umol \cdot kg^{-1} \cdot min^{-1})')
legend('Numerical Ra','Steeles Ra')

% Solve the ode with the minimized parameters
[t,Y] = ode45(@rhs_gly2,tspan,INITIALCONDITION,'',pars_min);

% Set errorbars for plotting
e = 0.031*glycerol;

```

```
% Plot the final solution
figure; clf;
plot(t,Y,'b')
hold on
plot(times,glycerol,'r',times,glycerol,'r*')
hold on
errorbar(times,glycerol,e,'r')
if sub_number == 0
    title({['Average Subject with pV: ' num2str(pV) ' and error: '
        num2str(m)], ['Sg: ' num2str(theta(1)) ', V: ' num2str(V)]})
else
    title({['Subject ' num2str(sub_number) ' with pV: ' num2str(pV) '
        and error: ' num2str(m)], ['Sg: ' num2str(theta(1)) ', V: '
        num2str(V)]})
end
xlabel('Time (mins)')
ylabel('Glycerol Concentration (Umol/L)')
legend('Numerical Solution','Data')

toc

end
% Sample data function
function [times,conc_MS,Ra_MS] = glycerol_data_sub1(pV)
% Here, we have times, conc, TTRback, and Ra

times =
    [-30,-20,-10,0,10,20,30,45,60,75,90,105,120,135,150,180,210,240];

conc_MS =
    [257.6973828,244.5264604,231.8942848,181.9609288,145.7852017,...
```

```
    192.9950518,160.8879589,129.7197223,95.74965022,123.4349839,110.0404136,...
    104.207144,155.0285876,102.1578241,123.7278908,109.6505151,111.0788612,...
    115.5340563];

TTRback =
    [0.493970959,0.726973362,1.012007115,1.635122289,2.896011204,1.531538257,...
    2.317223964,3.859970341,6.415936969,3.929360022,4.374384759,4.984151203,...
    2.316359451,4.332086755,3.051934093,4.121104944,3.860139364,4.631155272];

Ra_MS = CalcRa(0.1,pV,conc_MS,TTRback,times);

end

function ess = error_gly2(pars)
    global times glycerol tspan INITIALCONDITION

    [t,y] = ode45(@rhs_gly2,tspan,INITIALCONDITION,[],pars);

    ess = 0;

    for i = 1:length(times)
        for j = 1:length(t)
        if times(i) == t(j)
            diff(i) = glycerol(i) - y(j);
        end
        end
    end
```

```
    ess = norm(diff,2);

end

% RHS for the final solution
function dy = rhs_gly2(t,y,pars)
    global V t_vals %Ra_fit %Ra_basal I_fit I0

Ra = 0;

% pars = Sg Ra
% Idea: loop over the Ra_tvals to find where t is
% We need Ra_tvals(i) <= t <= Ra_tvals(i+1) for our Ra definition
for i = 1:length(t_vals)-1
    if ((t<t_vals(i+1)) && (t_vals(i)<t))
        alpha_1 = pars(i+1);
        alpha_2 = pars(i+2);
        t_diff = t_vals(i+1)-t_vals(i);
        Ra = alpha_1 + ((alpha_2 - alpha_1)/(t_diff))*(t - t_vals(i));
        break; % we found t; stop looking
    end
end

dy = -pars(1)*y + Ra/V;

end

function [C,Ceq] = nonlinfunction(pars)
% C(x) <= 0
% Ceq(x) = 0
global Ra_basal % Ra_area t_vals

```

```
C = 0;
Ceq = Ra_basal - pars(2);

end
```